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A STUDY OF CLINICAL AND METABOLIC PROFILE OFNEONATAL ENCEPHALOPATHY

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ABSTRACT

Background: The proportion of neonates with unexplained encephalopathy due to metabolic error are likely to be high in view of high consanguinity rates in our attending population. We therefore intend to evaluate this still undefined group of neonatal encephalopathy in neonates and evaluate the proportion contributedby inborn errors of metabolism. **Methods:** The cross sectional observational study was conducted in Department of Pediatrics Maulana Azad Medical College and associated LokNayak Hospital in collaboration with the Department of Radiology. **Results:** Of 200 babies, 10 neonates had IEM. Among the babies detected with IEM, 4 had congenital lactic acidosis, 1 each of isovaleric acidemia, methylmalonic acidemia, propionic acidemia, oroticaciduria, Tyrosinemia 1 and tyrosinemia 2. Among 10 cases, 3 patients found to have abnormal excretion of organic acids in urine. **Conclusion:** With the advent of the Golden minute NRP program and establishment of SNCUs with early registration and identification of ante-partum and post partum asphyxia the burden of HIE is bound to decrease. Metabolic causes both of transient nature and those due to inborn metabolic errors may then contribute to significant neonatal mortality and morbidity. Addressing this may improve our approach to attaining the Millennium Development goals. There is also the need to impart information in order to alter the dynamics of the thought process of both the neonatologists and pediatricians who despite concrete markers like a sibling death or consanguinity fail to carry bout metabolic testing. Apart from changes at the societal level identification of these disorders have important implication in each family as the diagnosis mayalter their reproductive decisions through genetic counseling.

KEYWORDS: NE, SNCU, HIE.

INTRODUCTION

Neonatal Encephalopathy (NE) is "a clinically defined syndrome of disturbed neurological function in the earliest days of life in the term infant, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, sub normal level of consciousness and often seizures".[1] Perinatal asphyxia is the most common cause in term babies followed by sepsis, intraventricular/intracranial haemorrhage, cerebral malformations, transient metabolic disturbances and inborn errors of metabolism (IEMs).[2,3] IEMs are rare as individual diseases, but as a group remain an important entity that present in neonatal age group.[4,5] The timely diagnosis and early initiation of specific therapy may be life-saving. It is important for neonatologists to keep in mind inborn errors of metabolism (IEMs) as a cause of illness in the neonatal period, as many disorders are treatable and, in most cases, successful outcome is dependent on a rapid diagnosis and early instigation of therapy.[6] Even with untreatable disorders, it is important to establish the diagnosis in the index case in order to allow prenatal diagnosis in subsequent pregnancies. In desperately sick neonates for whom no diagnosis is readily available, IEMs are near the top of the list of differential diagnosis. Typically, an IEM is suspected as a result of a suggestive combination of acute clinical symptoms without any prior warning.

Various simple preliminary laboratory tests can aid in diagnosis of IEM which can be further confirmed by advanced diagnostic techniques like tandem mass spectrometry and urinary gas chromatography and mass spectrometry.[7]

Since the investigative technologies are limited in the country the profile of metabolic errors contributing to neonatal encephalopathy are not completely elucidated. The proportion of neonates with unexplained encephalopathy due to metabolic error are likely to be

high in view ofhigh consanguinity rates in our attending population. We therefore intend to evaluate this still undefined group of neonatal encephalopathy in neonates and evaluate the proportion contributed by inborn errors of metabolism.

MATERIALS AND METHODS

The study was conducted in Department of Pediatrics Maulana Azad Medical College andassociated LokNayak Hospital in collaboration with the Department of Radiology.

STUDY DESIGN

The study was cross sectional observational study of one year duration.

Sample Size: The total number of neonates required was 200. This was based on an estimated prevalence of 5% with an precision of 2%, an alfa error of 5% with a power of 90%, sample size required was calculated as 193. Also, the average number of neonates with encephalopathy presenting to Ward 16 was calculated as 30/ month and average referrals to genetic clinic from other hospitals was 30/ month. This was deduced from the preceding year's data.

Study Population: This comprised of a population of

*All extramural neonates with encephalopathy referred to ward 16, Neonatal Intensive Care Unit, LokNayak Hospital.
*All neonates with encephalopathy referred to the Division of Genetics & Metabolism fromother hospitals including only those clinically evaluated.

SELECTION OF PATIENTS

Inclusion criteria

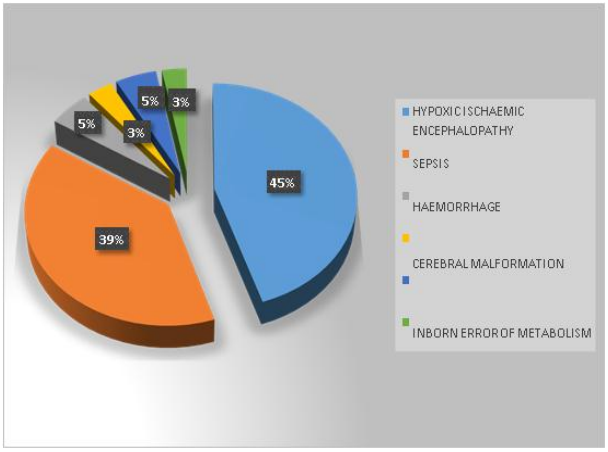
- All neonates with encephalopathy were recruited after they fulfilled the criteria defined as a clinically defined syndrome of disturbed neurological function in the earliest days of life in the term infant, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, sub normal level of consciousness and often seizures

- **Exclusion criteria**
- Neonates with reported encephalopathy on the referral slip but not clinically evaluated as the set criteria could not be evaluated.
- Neonates born to mothers with history of drug addiction/abuse

RESULTS

A total of 200 neonates (136 males and 64 females) aged 1-28 days were studied during the one year period. These included 11 neonates aged 1 day that is 24 hours of life, 83 aged 2-7 days and 106 aged 8-28 days. Among the neonates, 160 babies were admitted to neonatal intensive care unit, Lok Nayak Hospital. A total of 82 neonates were referred to the metabolic and genetic unit from other hospitals but only 40 were evaluated at the Division of Genetics & Metabolism and have been included. Out of these 30% neonates were delivered at home and 70% were delivered in other smaller primary hospitals and referred to a higher center for Level 3 SCNU care.

Hypoxic ischaemic encephalopathy was found to be the most common cause of encephalopathy diagnosed in 45% babies, sepsis in 39% haemorrhage in 5%, inborn error of metabolism in 5%, Cerebral malformation in 3%,transient metabolic disturbance (hypocalcemia, hypoglycemia) in 3%. Out of these three were referred with a diagnosis of HIE an7 were referred with a diagnosis of sepsis with meningitis or intracrainal hemorrhage. The diagnosis of HIE was made by history, clinical examination and USG findings. Sepsis work up (complete blood count, micro ESR, CRP, I/T ratio, blood culture) was done in patients with suspected sepsis. Transient metabolic disturbances were evaluated for serum glucose and calcium. Malformation and haemorrhage was made by neuroimaging which mainly included a bedside ultrasound followed by CECT wherever feasible Figure represents the etiological diagnosis in our cohort of neonates presenting with encephalopathy.



All neonates underwent estimation of lactate, ammonia, glucose estimation and calcium estimation. Ketones were initially checked by Ketostix. Serum lactate level was also found to be elevated in 30% of non IEM cases but they were found to be in shock Repeat lactate levels was found to be normal after the resolution of shock. Urinary GCMS and TMS was found to be normal in such cases. Serum Ammonia levels were found to be elevated in 20% of non IEM cases but urinary GCMS and TMS was normal. Two of these neonates with elevated ammonia were preterm and could have had THAN (transient hyperammonemia of the newborn). In all the neonates with diagnosed IEMS all had abnormalities in the basic profile except the neonate with MSUD. Fifty percent children with a confirmed IEM had raised serum ammonia levels and70% had high anion gap metabolic acidosis and 50%had ketosis. Forty percent had raised

lactate levels.

Of 200 babies, 10 neonates had IEM. Among the babies detected with IEM, 4 had congenital lactic acidosis, 1 each of isovaleric acidemia, methylmalonic acidemia, propionic acidemia, orotic aciduria, Tyrosinemia 1 and tyrosinemia 2.

Among 10 cases, 3 patients found to have abnormal excretion of organic acids in urine. Organic acidemia detected was propionic acidemia, isovaleric academia and methyl malonic acidemia. The clinical and laboratory features of patients with organic acidurias were NE, feeding difficulties vomiting metabolic acidosis, hyperammonemia, hypoglycemia, lactic acidemia, ketosis/ketonuria The ammonia levels were found to be high inall the three cases but the degree was variable. Mild hyperammonemia was found in methylmalonic academia(-120micromol/L) and moderate in isovaleric academia – (520micromol/L) while very high levels of ammonia was found in Priopionic Acidemia (2363micromol/L). All of these had presented after a normal period of around 7 days. The corresponding findings in these patients on Tandem Mass Spectrometry and Gas Chromatography are depicted in the table below

Case	Diagnosis	Findings on Tandem Mass Spectrometry	Findings on Gas Chromatography	MutationIdentified
One	Methylmalonic Acidemia	C3-10.2	Highly elevated levels of methylmalonic acid (26633 mmol/mol of Creatinine)	p.K621N and p.G648D inExon 12(compound Heterozygousform)
Two	Isovaleric Acidemia	C5-	Presence of Isovalerylgycine, elevated methylcitric acid, methylsuccinic acidand 3 methyloxaloacetic acid	Mutation testingnot done
Three	Priopionic Acidemia	C3-13.8	Presence of Priopionyl carnitine, methylcitric acid	c.229C→T (p.R77W) (homozygous form)

DISCUSSION

Prospective data for Neonatal Encephalopathy is scarcely available in published literature. Inborn errors of metabolism have been thought to be very rare in India. Only few studies are available and are individual case based and none till date has focused on patients presenting in the newborn period with a specific prototypic presentation. The aim of the study was to determine the proportion of neonates having an inborn error of metabolism(IEM) presentingas neonatal encephalopathy. The study was carried out in a tertiary care hospital in New Delhi. Two hundred babies were recruited in our referral center which primary caters to be extramural neonates with birthing being either at home or in some other primary center. These neonates were referred in view of eitherun explained cause or requirement for a tertiary level care/ventilation.

The major contributors of neonatal encephalopathy in our study were hypoxic ischaemic encephalopathy (45%), sepsis (39%), intraventricular haemorrhage (5%), cerebral malformation(3%), transient metabolic disturbances(3%). apart from 5% being due to an inborn error of metabolism. Tekgulet al.[8] reported a series of 116 symptomatic neonates with seizures, without categorizing whether they had encephalopathyor not, over a three-year period and identified global hypoxic ischemia, cerebral vaso-occlusive diseases (perinatal arterial strokes) and intracranial haemorrhages to be the most common etiological factors. Central nervous system infections and metabolic disturbances were less commonly noted in this series. In another study conducted in Iran over a period of three years from September 2008 to September 2011 in the same category (neonates with seizures) were evaluated for etiology. The main etiology identified included hypoxic-ischemic encephalopathy (34.3%), infections (24.4%), intracranial hemorrhage (6.9%), hypoglycemia (5.9%), hypocalcemia (2.9%), inborn error of metabolism (1%), and unknown cause (24.5%). The proportion of patients identified with IEM were reported to be lower than our study. The limiting factors could have been low index of suspicion, no algorithm of evaluating every newborn with a set of predesigned investigations and limited resources to evaluate metabolic errors though these were not outlined in the study.

A study was reported by the metabolic division of a tertiary Hospital in Muscat, Oman,[9] over a seven-year period from June 1998 to May 2005. One hundred and sixty six high-risk neonates were screenedfor IEM who had at least one major risk factor for a high index of suspicion of IEM. IEM were detected in 38 babies (23%). The reported incidence differs remarkably from our study. In our study the recruitment was unbiased. No risk factors for IEM was needed for inclusion as we wanted to study the overall prevalence of IEMS, which could masquarade as NE. Also the duration of study was one year and the consangunity rate must have definitely been lower than that existing in that area where matings are generally consanguinous. The exact rate of inbreeding or endogamous mating was not clearly outlined. In our study the history of consanguinity was found in 8(80%) families. Of the degrees of

consanguinity first-cousin marriages were present in 80% while 20% were born to second cousin matings. Another study conducted at Islamabadbetween January 2006 and June 2011 also evaluated the consanguinity in the population whose neonates were classified to have an IEM. Of the 10patients diagnosed with IEM, 6 were neonates. History of consanguinity was found to be in all patients(100%). In our country where consanguinity is common,the likely strong indicator in the absence of universal newborn screening for inborn metabolic errors should be succinct evaluation of a family tree or heredogram for the type of mating.

Death of siblings with similar complaints was another outstanding finding that has also been reported by other studies. A history of death of the similarly affected sibling (death without an identified cause) was elicited in 7 families (70%) This high number of death of siblings in our setup indicates that it is a very significant marker to be alert about the possibility of inborn errors of metabolism and that there is a delay in diagnosis of such diseases in our setup. In a study from Karnataka 50 children were evaluated for IEM history of sibling deaths were found to be in 15% cases. Our study reiterates that where newborn screening is not a common place, this may be used as a strong predictor to initiate testing for IEM.

Regarding the age of presentation none of the IEM babies presented within first 2 days of life. The average age at the symptom onset was 8 days and the mean gap between symptom onset and diagnosis was 7 days. The minimum age of presentation was 3 days. Once admitted to our hospital the time to attaining a diagnosis was an average of 72 hours. All babies were found to be normal during initial days of birth. Antenatal period and delivery period were normal. This is understandable as in utero the placenta dialyzes the metabolites and neonates have low levels in the initial phase of life till feeding is well established. In the study from Muscat the median age at presentation was 7 days but included a wide range till 28 days of life.

Total of 10 neonares were identified with an IEM giving the proportion to be 5%. Out of the spectrum of disorders identified the IEMs diagnosed belonged to the small molecule group of disorders. The predominant group of IEM included disorders of energy metabolism(4 babies were diagnosed as congenital lactic acidosis), organic academia (3 cases each of propionic acidemia,isovaleric acidemia,methylmalonic acidemia), aminoacidopathies (1 with Tyrosinemia type 1 and one with Tyrosinemia type 2) and1 with Maple syrup urine disease which was belongs to both the categories

As expected the clinical manifestations were non-specific. Shefa et al[10] in a study conducted at Islamabad found that among IEM seizures and respiratory distress were found to be most common. Similarly in our study, the inclusion criteria itself was a neurologic state of altered sensorium with or without seizures. The additional clinical presenting features included vomiting and feeding difficulties and fast breathing. The fast breathing could have been consequent to the underlying state of acidosis. The neonate identified with Tyrosinemia type 1 had additional features of liver failure(like jaundice, melena and abdominal distension) while the one identified with Tyrosinemia type 2 had corneal ulcers and painful hyperkeratotic plaques on the palms and soles. All of these cases had been referred to with a label of HIE (3/10) or as sepsis with meningitis/hemorrhage (7/10) and in none a diagnosis of IEM was even thought of.

Neonatalencephalopathy rapidly progresses to fatality. Out of 10 neonates, 9 neonates expired before the diagnosis was made. The neonates with congenital lactic acidosis died within 72 hours while death in organicacidemia group occurred variably after 5 days, InT yrosinemia Type I and MSUD neonates death occurred after 10 days. The infant with tyrosinemia type 2 is on follow up and is on low phenylalanine and tyrosine diet.

Screening tests like blood gas analysis, estimation of lactate and ammonia levels, urine examination for ketones provide valuable clues to the underlying metabolic disease. In biochemical labs results basic metabolic testing was extremely useful in contributing to the final yield of a confirmatory case. Fifty percent children with a confirmed IEM had raised serum ammonialevels and70%had high anion gap metabolic acidosis and 50%had ketosis. Forty percent had raised lactate levels.The introduction of GCMS, and tandem mass spectrometry (MSMS) substantially helped in determining the etiology. The same techniques were employed to diagnose our patients.

Many studies have shown that OAs are relatively common disorders among the IEMs presenting in the neonatal period. In our study congenital lactic acidosis was the most frequently diagnosed followed by organic academias, and amino acid disorders. It is noteworthy that not a single neonate with a fatty acid oxidation disorder or galactosemia was detected in the population studied; This could be duetoany of the following reasons It is known that some of the patients among this group of disorders can present with sudden death infant syndrome and they die before diagnosis is made and a low prevalence of these disorders has been documented in some of the screening programs. In a study at a tertiary care hospital in Karachi the prevalence of selected disorders of inborn errors of metabolism by performing selective screening of high risk clinically suspected individuals was done. It was found that 62.5% had organic acidemias, 6.2% had Ornithine Transcarbamylase (OTC) deficiency (Urea cycle defect) and 31.2% had congenital lactic acidemias. The proportion of Congenital Lactic acidosis (mitochondrial group) is similar to that found in our study with our

patients having 40%. Incidence or prevalence of congenital lactic acidemias are unknown But unfortunately further subtypes like the class of mitochondrial disorders could not be diagnosed due to lack of resources.

Of the neonates presenting with congenital lactic acidosis, 2neonates presented within 7 days while other two after 7 days. None of these neonates had evidence of poor perfusion and all these neonates presented with tachypnea. All showed highly elevated levels of lactic acid(>1000)in urinary GCMS The mortality in this group was 100%. Mitochondrial gene sequencing could not be done due to unaffordability in this group as well as the poor outcome which was demotivating for the parents.

In our study among organic academia 1 case each of propionic acidemia, methylmalonicacidemia and isovaleric acidemia was found. Clinical features were similar in all cases. Mildhyperammonemia was noted in the neonate with methylmalonic acidemia and isovalericacidemia while very high levels were seen in the neonate with propionic acidemia. These were identified on the basis of utilizing both the modalities TMS and GCMS. Deficiency of propionyl-CoA carboxylase results in the disease known as propionic acidaemia, which is characterized by accumulation of propionic acid and the production of a wide range of abnormal metabolites, in particular, 3-hydroxypropionate, methylcitrate and propionyl glycine. Our patient demonstrated the classic snapshot with elevation of the both 3-hydroxypropionate, methyl citrate along with the presence of Prionylglycine. Mutation testing revealed a known pathogenic mutation previously reported in literature. Both the parents were carriers for the samemutation.

Methylmalonicacidaemia gives rise to a range of secondary effects similar to those of propionic acidaemia – hyperammonaemia, ketosis, and inhibition of glycine catabolism. This has been the commonest organic acidemia reported from the Indian subcontinent. These levels are also elevated in neonates born to mothers with B12 deficiency but surprisingly we did not find this in any neonate with NE. One reason for this could be the strict inclusion of neonates with NE but not of asymptomatic neonates. Mutation Testing revealed a compound heterozygous state of 2 novel mutations p.K621N and p.G648D in Exon 12(compound Heterozygous form)The mutation was evaluated to be pathogenic by all the 3 mutation testing software's i.e., Polyphen, SIFT and mutation taster.

Isovalericacidaemia is a defect of leucine catabolism caused by deficient activity of the enzyme isovaleryl-CoA dehydrogenase. This leads to intracellular accumulation of isovaleryl-CoA, with the appearance of the characteristic metabolite isovalerylglycine in body fluids. We could not test the pathogenic mutation in this neonate.

The overall prevalence of organic acidemia is not available in the cohort with NE. The incidence of these can be prospectively evaluated only from countries with expanded newborn screening.

Methylmalonicacidemia was found by the Massachusetts urine-based neonatal screening programme was 1:48,000but, with less-severe cases included, may be as high as 1:25,000. In the Massachusetts screening programme, only one patient with propionic acidemia was found amongst 331,143 infants screened. Data from the West Midlands Region over a 5-year period suggest that the incidence for organic acidemia collectively is at least 1:15,000 live births and, for methylmalonicacidemia about 1:50,000.

One case each of tyrosinemia type 1 and 2was found. Tyrosinaemia type I is caused by a deficiency of fumarylacetoacetase. These primary metabolites are converted to succinylacetoacetate and then to succinylacetone which is excreted in large amounts in urine. The excretion of succinylacetone, forms the basis of diagnosis. In our case liver failure and consequent NE brought the neonate to medical attention. The highly elevated AFP levels with significant bleeding also served as a vital clue in this neonate Tyrosinaemia type I occurs world- wide, but the frequency is variable. In the SaguenayLac-St-Jean region of northeastern Quebec, Canada, it is particularly common with an incidence of 1:1846. In most other countries the frequency is closer to 1 in 100,000. In our study, the patient with tyrosinemia type 1 presented with failure-to-thrive, hepatomegaly, jaundice, and gastrointestinal bleeding while baby with tyrosinemia type 2(oculo-cutaneoustyrosinemia) presented withcorneal ulcers and painful hyperkeratotic plaques on the palms and soles. Large elevations in serum transaminasestogether with metabolic acidosis and electrolyte disturbances due to renal tubular dysfunction was noted in Tyrosinemia type 1. High levels of tyrosine(>500 micro mol/L) in TMS was observed in both cases.Both were differentiated by elevated levels of succinylacetone in urine in Tyrosinemiatype baby with tyrosinemia type 1 died after 7 days while baby with tyrosinemia type 2 is on follow up and is put on low phenylalanine and tyrosine diet.

One neonatewas diagnosed with maple syrup urine disease. The baby presented at day 10 of life after a normal period with lethargy, poor feeding, vomiting, seizures, and loss of consciousness. The neonate had a known mutation in the homozygous state of homozygous state of c.1016 C>C/T (amino acid change p.S339L) in Exon 9 of the BCKDHB gene in Exon 9 of the BCKDHB gene BCHKD B gene. Both the parents were carriers for the same mutation Maple syrup urine disease is caused by an inherited deficiency of the branched-chain 2-keto acid dehydrogenase, resulting in a marked increase in plasma and urinary concentration of branched- chain ketoacids and the corresponding branched-chain aminoacids, leucine, isoleucine and

valine. The prevalence of this disorder is again by prospective newborn screening data. In a review of over 9 million neonatal screening tests performed worldwide over a 12-year period, the combined incidence of classical and intermediate forms of disease was found to be 1:224000 births.

CONCLUSION

With the advent of the Golden minute NRP program and establishment of SNCUs with early registration and identification of ante-partum and post partum asphyxia the burden of HIE is bound to decrease. Metabolic causes both of transient nature and those due to inborn metabolic errors may then contribute to significant neonatal mortality and morbidity. Addressing this may improve our approach to attaining the Millennium Development goals. There is also the need to impart information in order to alter the dynamics of the thought process of both the neonatologists and pediatricians who despite concrete markers like a sibling death or consanguinity fail to carry bout metabolic testing. Apart from changes at the societal level identification of these disorders have important implication in each family as the diagnosis may alter their reproductive decisions through genetic counseling.

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