Original Research Article

THIAMINE DEFICIENCY IN EXCLUSIVE BREASTFED INFANTS WITH ENCEPHALOPATHY ATTENDING GB ¿¿PANTH CHILDREN HOSPITAL SRINAGAR.

Abstract:-

Introduction:-Thiamine deficiency has historically affected countries and populations consuming milled white rice. Thiamine deficiency in infants can have acute presentation of encephalopathy with shock with severe metabolic acidosis and death sometimes, if not promptly treated with intravenous dose of thiamine.

Aims:-To study the biochemical deficiency of thiamine in exclusively breast fed infants presenting with encephalopathy and compare them with age matched controls and to study their clinical course and short term outcome (till discharge).

Materials and methods:-After dividing infants into 4 groups on the basis of age in days: (31-60 days; 13 in cases and 8 in controls; 61-90; 4 in cases and 3 in controls; 91-120 days; 2 in cases and none in controls; and >120 days 4 each in cases and controls. This study primarily included selection of case/control subjects. Two case control analysis were conducted. In the first one, blood thiamine levels were compared between infants with encephalopathy and without encephalopathy. In second one, breast milk thiamine levels were compared between infants with encephalopathy and without encephalopathy. Students independent t-test was used for statistical analysis.

Results:-Out of 38 infants 23 had presented with encephalopathy and 15 were healthy taken as controls. The mean blood levels of thiamine in infants with encephalopathy in cases was 17.29nmol/l with a Standard deviation of 8.86 the levels ranged between 13.47 and 21.13.The mean value of controls was51.31 with a Standard deviation of 25.52 ranged between 23.25 and 124.7. The P value was <0.001 and was considered statistically significant. The ROC analysis of the data obtained from thiamine levels obtained in study patient's blood compared with control group.

Conclusion:-Thiamine deficiency can be clinically and biochemically attributed to presentation of infants with acute encephalopathy.

Keywords: Thiamine levels, Breastfed infants, Encephalopathy, Metabolic acidosis.

Introduction

Thiamine (Vitamin B1) is an essential micronutrient with dual co enzymatic and non-co enzymatic functions. It is involved in carbohydrate and branched-chain amino acid metabolism, as well as in the production of neurotransmitters, myelin, and nucleic acids[1,2,3,4]. Inpaediatrics, the overall clinical picture of thiamine deficiency (TD) is not easy to recognize, mimicking or being confused with other diseases. Not surprisingly, the likelihood of misdiagnosis of TD is even greater in

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Comment [U2]: . ResearchPapersand Short Notes should follow thestructureof Abstract, Introduction, Methodology, ResultsandDiscussion, Conclusion, Acknowledgements, CompetingInterests, Authors' Contributions, Consent (whereapplicable), Ethicalapproval (whereapplicable), andReferences plus figures and/ortables.)

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Abstract
The abstract shouldbeconciseandinformative. It shouldnotexceed 300 words in length. It shouldbrieflydescribethepurposeofthework, techniquesandmethodsused, major findingswithimportant data andconclusions. Differentsub-sections, as givenbelow, shouldbeused. No referencesshouldbecited in this part. Generally non-standard abbreviationsshouldnotbeused, ifnecessarytheyshouldbeclearlydefined in the abstract, atfirst use.

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Comment [U4]: SAMPLE ABSTRACT:
Aims: Hereclearlywritetheaimsofthisstudy.
Sample: To correlate plateletcount, splenic index (SI), plateletcount/spleendiameterratioand portal-systemicvenouscollateralswiththepresenceofesophagealvarices in advancedliverdiseasetovalidateotherscreeningparameters.
Study design: Mentionthe design ofthestudyhere.
PlaceandDurationofStudy: Sample: Departmentof Medicine (Medical Unit IV) andDepartmentofRadiology, Services Ins ... [1]

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resource-limited settings[5,6,7]. Despite being easily treatable, TD continues to be seen in all age groups in both high and low resource countries with potentially severe and life-threatening consequences[8,9,10,11]. Thiamine deficiency global prevalence is poorly documented. It principally affects precarious communities where children are most vulnerable and where dietary habits rely on refined processed cereals or tubers (e.g., rice, wheat, cassava).

The earliest presentation of TD in breastfeeding infants up to 3 months of age include non-specific signs like loud piercing cry and colic and eventually edema, cyanosis, unexplained metabolic acidosis ophthalmoplegia, nystagmus, encephalopathy, lactic acidosis and congestive heart failure may appear[6,12,13-18]. If undetected at this stage, death can occur within hours but prompt recognition and treatment with injectable thiamine can rapidly reverse the clinical picture and drastically improve the prognosis. Later, at 4–7 months, the infant is more likely to present with an aphonic form. After increasing cough and dyspnea, the cry changes from hoarse to soundless ("aphonic cry"). Similar to the younger infant, without treatment this condition can evolve into severe acute congestive heart failure, edema, respiratory distress, and eventually death within a few days[18] .Acute Infants who are exclusively breastfed are dependenton breast milk for all their nutritional requirements. Hence thiaminedeficiency in breastfed infants can be taken as an index for the prevalenceof thiamine deficiency in a community or population.

In our state, many patients in the age group 1 to 6 months have been presenting to us with unexplained severe metabolic acidosis with encephalopathy of acute onset. With the dietary practices, and the biological plausibility of thiamine deficiency in such patients, patients have been empirically treated with thiamine supplementation as the first line treatment. The response to such treatment has been quick and complete. No scientific study with detection of thiamine levels in patients is available in the valley which was the reason to carry out this study. The aim of this study is to identify such patients and draw their blood samples along with their mother's breast milk samples and look for thiamine deficiency.Ok,ok

Materials and method:- This study primarily included selection of case/control subjects from the Department of Paediatrics, GB Panth Children Hospital Srinagar an associated hospital of Govt. Medical College, Srinagar from August 2017 to July 2019 in collaborative efforts with Biochemistry and Molecular Biotechnology Laboratory (in collaboration with ICMR) Division of Basic Sciences, SKUAST-K, Shalimar campus.Our study was approved by the Ethical Committee of Government Medical College Srinagar via communication No. 130/ETH/GMC/ ICMR; dated 19-03-2016 and written consent forms were signed by the parents before participating in the study.

Laboratory investigations included breast milk and blood samples for estimating thiamine levels and its phosphate esters in both cases and controls. Other routine investigations include blood counts, serum biochemistry and electrolytes, blood sugar and blood gasses. CSF examination was done in suspected cases of meningitis. Other investigations included blood

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ammonia levels, Tandem mass spectrometry, Urine gas chromatography, Mass Spectrometry and radiological investigations (MRI, Cranial USG) were done in some of the patients. Detailed epidemiological, dietary, clinical, laboratory and treatment data of patients were obtained. Age matched exclusive breast fed infants admitted in hospital and who needed sampling for reasons other than encephalopathy served as controls. All infants admitted to us after getting blood sample collected received IV thiamine. Their clinical progress was assessed from time to time as per the diagnostic criteria of encephalopathy.

Inclusion Criteria:-All exclusively breastfed infants of age one month or above who presented with unexplained encephalopathy along with their breast fed mothers and exclusively breast fed infants admitted in hospital for conditions not associated with encephalopathy served as controls.

Exclusion criteria:-Infants in whom alternative etiology of encephalopathy was established on clinical history, examination and investigations, Infants of age less than one month and Infants on formulae.

Statistical analysis:- The recorded data was compiled and entered in a Spreadsheet (Microsoft Excel) and then exported to data editor of SPSS version 20.0(SPSS Inc., Chicago, Illinois, USA). Continuous variables were exported as Mean+-SD and categorical values were summarized as frequencies and percentages. Graphically the data was presented by Bar and Pie diagrams. Shapiro-Wilk Test and Normal Probability Plot were used to test for normality of data. Students Independent T Test were used for comparison of continuous variables. Chi-Square Test/Fishers Exact Test, wherever appropriate were employed for comparison of categorical variables. ROC analysis was employed to determine diagnostic accuracy of optimal cut off for thiamine levels in baby's blood and corresponding mothers lactating milk for predicting encephalopathy in patients.

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ROC

Results:-Our study consisted of total of 38 patients, among whom males and females comprised of 26 and 12 respectively. Patients were classified into four groups on the basis of age as shown in [Table-1]

Table 1: Age distribution

Age (Days)	Cases		Controls		P-value
	No.	%age	No.	%age	
31-60 Days	13	56.5	8	53.3	0.921
61-90 Days	4	17.4	3	20.0	
91-120 Days	2	8.7	0	0.0	
> 120 Days	4	17.4	4	26.7	
Total	23	100	15	100	
Mean±SD (Range)	85.4±47.39 (31-180)		83.7±54.04 (35-180)		

In our study the mean blood levels of thiamine in cases was 17.29nmol/l with a Standard deviation of 8.86, the levels ranged between 13.47 and 21.13.The mean value of controls was 51.31 with a Standard deviation of 25.52 ranging between 23.25 and 124.7. The P value was <0.001 and was statistically significant as depicted in [Table-2]

Table2: Comparison based on thiamine levels in baby's blood between cases and controls

Group	Mean	SD	Range	95% CI	t-value	P-value
Cases [n=23]	17.29	8.86	3.46-38.56	13.47-21.13	5.539	<0.001*
Controls [n=15]	51.31	27.52	23.25-124.7	36.07-66.55		

Statistically Significant Difference (P-value <0.05)

In our study the mean thiamine levels in mother's breast milk in cases was 108.16mcg/l with a standard deviation of 58.93, and a range between 16.33 and 214.92 in cases. Thiamine levels in breast milk in controls revealed a mean of 252.68mcg/l, with a standard deviation of 66.70, and a range of 159.33 and 361.2. The 95% Confidence Interval was 82.68-133.65 in cases and 215.74-289.62 in controls. T value was 5.53 and P value was <0.001 & was statistically significant as shown in [Table-3]

Table 3: Comparison based on thiamine levels in mothers breast milk between cases and controls

Group	Mean	SD	Range	95% CI	t-value	P-value
Cases	108.16	58.93	16.33-214.92	82.68-133.65	5.539	<0.001*
Controls	252.68	66.70	159.33-361.2	215.74-289.62		

*Statistically Significant Difference (P-value <0.05)

Table-4 shows ROC analysis of the data obtained from thiamine levels obtained in study patient's blood compared with control group. In this study if we take a cut off level of 25.14 for predicting thiamine responsive encephalopathy in patients, a sensitivity of 86.9%, specificity of 93.3%, a positive predictive value of 95.2%, predictive value of 82.4%. An overall accuracy of 89.5% was obtained a negative p value was 0.001

Table:-4 Diagnostic accuracy of thiamine levels in babies blood for predicting thiamine responsive encephalopathy

	Value	95% CI
Optimal Cut-off	d 25.14	
Sensitivity	86.9	66.4-99.72
Specificity	93.3	68.1-99.9
Positive Predictive Value (PPV)	95.2	76.2-99.99
Negative Predictive Value (NPV)	82.4	56.7-96.2
Accuracy	89.5	
Area under the curve	0.962	0.844-0.997
P-value	<0.001	

Table-5 shows diagnostic accuracy of thiamine levels in mother's breast milk for predicting thiamine responsive encephalopathy in their infants. At a cut off value of 153.7 a sensitivity of 82.6%, a specificity of 100 percent, a positive predictive value of 100%, a negative predictive value of 78.9 was obtained. An accuracy of 89.5 was obtained. The p value was less than 0.001.

Table 5: Diagnostic accuracy of thiamine levels in mothers breast milk for predicting thiamine responsive encephalopathy in their infants

	Value	95% CI
Optimal Cut-off	d 153.7	-
Sensitivity	82.6	61.2-95.1
Specificity	100	78.2-100
Positive Predicted Value (PPV)	100	82.4-100
Negative Predicted Value (NPV)	78.9	54.4-93.9
Accuracy	89.5	-
Area under the curve	0.962	0.844-0.997
P-value	<0.001	-

Statistical analysis:- The recorded data was compiled and entered in a Spreadsheet (Microsoft Excel) and then exported to data editor of SPSS version 20.0(SPSS Inc., Chicago, Illinois, USA). Continuous variables were exported as Mean+-SD and categorical

values were summarized as frequencies and percentages. Graphically the data was presented by Bar and Pie diagrams. Shapiro-Wilk Test and Normal Probability Plot were used to test for normality of data. Students Independent T Test were used for comparison of continuous variables. Chi-Square Test/Fishers Exact. Test, wherever appropriate were employed for comparison of categorical variables. ROC analysis was employed to determine diagnostic accuracy of optimal cut off for thiamine levels in baby's blood and corresponding mothers lactating milk for predicting encephalopathy in patients.

Discussion:-Thiamine deficiency has historically affected countries and populations consuming milled white rice. Polished rice is the staple diet in Kashmir.. Infants who are exclusively breastfed are dependent on breast milk for all their nutritional requirements. Hence thiamine deficiency in breastfed infants can be taken as an index for the prevalence of thiamine deficiency in a community or population. Exclusively breastfed infants between 1-6 months of age who presented with unexplained encephalopathy and with severe acute life threatening metabolic acidosis were included in study. The statistical analysis revealed that cases and controls did not differ significantly in age and sex The mean age of presentation for cases in our study was 86 days which was in similarity to mean age of presentation 95 days as reported by **Bhat JI et al (2017)**[19] and 51 days as reported by **Qureshi U et al (2016)**[20]. The most common symptom observed in our study was decreased feeding noted in 87% of the patients. Other symptoms were lethargy in 70%, irritability in 14%, moaning in 52.2%, vomiting in 10%, fast breathing in 30.4%, constipation like non-specific symptom in 13% and seizures in 9%. The presenting symptoms were in similarity to symptoms reported by **Qureshi UA et al (2016)**[20], **Bhat JI et al (2017)**[19], **Kornreich L et al (2005)**[22]. The most common signs at admission in our study were shock in 65% patients, acidotic breathing in 48%, vacant stare in 43% and gasping respiration in 39% of the study group. The investigations revealed a Hb. of 8.6-13.9g/dl with a mean 10.76 and a standard deviation of 1.58. Sodium was 129-157mmol/l with a mean of 142.1 and a standard deviation of 7.13. Potassium was 3.1-5.1mmol/l with a mean of 4.07 and a standard deviation of 0.49. Lactate levels ranged from 5mmol/l to above detectable limits. pH ranged from below detectable limits to 7.3. Similar reports were obtained by **Qureshi UA et al (2016)**[20]who reported a mean Hb of 9.1, mean Na of 137meq/l, Mean K of 4.3, Lactate levels 5->15, pH was <6.8-7.2. Cranial ultrasonography was done in all cases and 11 out of 23 (47%) patients were observed to have findings consisting with **Wani NA et al (2016)**[21] who found hyperechoic appearance of basal ganglia. Thiamine Diphosphate (TDP) in whole blood and Thiamine Monophosphate (TMP) in corresponding mother's milk was analysed by HPLC. A threshold for blood thiamine TDP and milk thiamine TMP was taken as 65 nmol/l and 300 nmol/l respectively. Methods and cutoffs were consistent to the methods and cutoffs demonstrated by **Stuetz W et al (2012)**[23]. Our study revealed that thiamine levels (TDP) in cases were low with a mean of 17.29 nmol/L with a standard deviation of 8.8nmol/L, range was 3.46-38.56nmol/L as shown in table-2. Levels in controls had a mean of 51.31nmol/L and a standard deviation of 27.52nmol/L, with a range of 23.25-124.7nmol/L as shown in table-2. Similar deficient state of thiamine was found in

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cases by **Qureshi UA et al (2016)**[20] with mean blood thiamine levels of six infants 41nmol/L with a range of 11-69 nmol/L (control 78–185 nmol/L). Significant difference in thiamine levels between cases and controls was found by **Keating EM et al. (2015)**[24] who recording levels below estimated levels of normal (17nmol/l) in 43% cases and 34% controls. **Bhat JI et al (2017)**[19] also noted mean blood thiamine levels (TDP levels) of 5 patients with a drastic decrease (10-49nmol/l). We observed that thiamine levels in mother's milk in cases was 16.33- 214.92 with a mean of 108.16 and a standard deviation of 58.93 as shown in Table-3. Thiamine levels in controls was 159.33-361.2 with a mean of 252.68 and a standard deviation of 66.70. Levels differed significantly between cases and controls as shown in table-3. Similarly results regarding breast milk deficiency in thiamine was found by **Stuetz W et al (2012)**[23] who reported thiamine deficiency in (4%) mother's milk in antenatal clinics in Maela refugee camps (levels <300). All patients were given IV Thiamine bolus (100mg IV) along with supportive care and all patients improved. Similar response to thiamine was observed by **Bhat JI et al (2017)**[19]. Mothers were also treated with thiamine orally for a period till exclusive breast feeding continued. **Bowman BA et al (2013)**[25] in their study found only modest improvement in thiamine status in infants after thiamine supplementation in mothers and hence thiamine supplement needs to be given to infants as well. Clinical improvement was observed though repeat thiamine levels were not conducted in our study due to financial constraints. We followed a long term outcome in 12 cases who were followed 4 months after their admission and all were found to have no recurrences and no neurological or cardiac symptoms. Similar long term good prognosis was demonstrated by **Ornoy A et al (2013)**[26].

Limitations:-Thiamine levels of only 23 cases and 15 controls were done due to financial constraints, which could only give a crude idea of the magnitude of thiamine deficiency in the general population. Analysis of thiamine levels of large number of patients could have revealed better information about the mode of presentation, clinical signs on examination and at what threshold level we can expect such life threatening emergencies.

Conclusion:- Thiamine deficiency can be clinically and biochemically attributed to presentation of infants with acute encephalopathy. Treatment of patients with high dose thiamine at the time of presentation can cure acute life threatening metabolic acidosis and large thiamine supplement should be given to the population so that such deficiency is corrected. Mass awareness regarding healthy cooking practices and knowledge about thiamine rich foods needs to be adopted.

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SAMPLE ABSTRACT:

Aims: Hereclearlywritetheaimsofthisstudy. Sample: To correlate plateletcount, splenic index (SI), plateletcount/spleendiameterratioand portal-systemicvenouscollateralswiththepresenceofesophagealvarices in advancedliverdiseasetovalidateotherscreeningparameters.
Study design: Mentionthe design ofthestudyhere.
PlaceandDurationofStudy: Sample: Departmentof Medicine (Medical Unit IV) andDepartmentofRadiology, Services Instituteof Medical Sciences (SIMS), Services Hospital Lahore, betweenJune 2009 and July 2010.
Methodology: Pleasewritemain points oftheresearchmethodologyapplied. Sample: Weincluded 63 patients (40 men, 23 women; age range 18-75 years) withlivercirrhosisand portal hypertension, withorwithoutthe medical historyof gastrointestinal bleeding. Clinical as well as hematologicalexamination (plateletcount) andultrasonography (gray as well as color Doppler scaleincludingsplenic index andsplenorenal/ pancreaticoduodenalcollaterals) wasdonebesidesupper GI endoscopy for esophagealvarices. Plateletcount/spleendiameterratiowasalsocalculated.
Results: Kindly make sureto include relevantstatisticshere, such as sample sizes, response rates, P-valuesorConfidenceIntervals. Do notjustsay "thereweredifferencesbetweenthegroups". sample: Out of 63 patients, 36 patientswithsmallvarices (F1/F2) and 27 withlarger (F3) varicesweredetectedonendoscope. Significantincrease in meansplenic index fromlow (86.7 +/- 27.4) to high (94.7 +/- 27.7) grade variceswasdocumented. Oppositetrendwasfoundwithplatelets (120.2 +/- 63.5 to 69.8 +/- 36.1) andplatelets/ splenicdiameterratio (1676.7 to 824.6) decliningsignificantly. Logisticregressionshowedspleniccollateralsandplatelets are significantlybutnegativelyassociatedwithesophagealvarices grades.
Conclusion: Non-invasiveindependentpredictors for screeningesophagealvaricesmaydecrease medical as well as financial burden, henceimprovingthe management ofcirrhoticpatients. Thesepredictors, however, needfurtherworktovalidatereliability.