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Measurement of Arginase Activity in Erythrocytes in Newborns and Children and its Correlation with Plasma Ammonia Concentration

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Abstract

Arginase is a urea cycle enzyme which catalyzes the cleavage of L-arginine to L-ornithine,and urea. Arginase deficiency is inherited as an autosomal recessive genetic disorder. Hyperammonemia refers to a condition with elevated levels of ammonia in the blood, which is a product of protein degradation. The lack of the arginase enzyme results in excessive accumulation of nitrogen, in the form of ammonia (hyperammonemia) and arginine (hyperargininemia) in the blood. In the present study, the erythrocyte arginase activity is measured along with plasma ammonia concentration in the newborns and children. The study group consists of 133 subjects which are divided into two groups based on the ammonia level. Group 1 consists of subjects with normal ammonia (n=92) and Group 2 consists of subjects with high ammonia (n=41). We found a significant decrease in arginase activity in the high ammonia group compared to the normal ammonia group. A significant negative correlation between arginase and ammonia is observed in both the groups. The result of this study suggests that arginase deficiency could be the cause for hyperammonemia in these cases. Hence, we suggest that estimation of erythrocyte arginase activity can be used as a screening procedure to detect arginase deficiency in newborns, infants, and children with hyperammonemia.

Keywords: Arginase, hyperammonemia, urea cycle, newborn screening.

Introduction

Arginase is a urea cycle enzyme which catalyzes the cleavage of L-arginine to L-ornithine, and urea. Arginase exists in two isoforms[1]. Arginase-1 is a cytosolic protein, expressed primarily in the liver and to some extent in the erythrocytes[2]. It is believed to be chiefly responsible for ureagenesis, nitrogen homeostasis and is the one missing enzyme in hyperargininemia patients. Erythrocyte arginase activity is one of the sources for ornithine present in plasma. The availability of ornithine may be important for peripheral tissues such as cartilage and bone since these tissues have low or no arginase activity[3]. Arginase-2 is a mitochondrial protein, expressed in many extrahepatic tissues, such as the brain, spinal cord, kidney, small intestine, and mammary gland, but not in mature erythrocytes[4]. Hyperargininemia is caused by the deficiency of the Arginase I, which is a treatable inborn error of the urea cycle. This condition rarely presents in the newborn period, and hyperammonemia is not a common biochemical hallmark[5]. This condition is not associated with a hyperammonemic encephalopathy in the neonatal period. It typically presents later in childhood between 2 and 4 years of age with predominantly neurological features. If untreated, it may lead to neurological disorders[6]. Arginase deficiency is inherited as an autosomal recessive genetic disorder. Hence, screening newborn babies for arginase deficiency will be helpful for the early diagnosis of the disease and subsequent management.

Hyperammonemia

Hyperammonemia refers to a condition with elevated levels of ammonia in the blood, which is aproduct of protein degradation.Primary hyperammonemia (congenital hyperammonemia) - caused by several inborn errors of metabolism that are characterized by decreased activity of any of the enzymesin the urea cycle of an individual[7]. The severe hyperammonemia observed in other urea cycle defects is rarely observed in patients with arginase deficiency for at least two identifiable reasons. The first reason is that formed arginine, which contains two waste nitrogen molecules, can be released from the hepatocyte and excreted in urine. The second reason may be attributed to the inducibility of the Type II isozyme in peripheral tissues, which can attack the arginine released by the hepatocyte and produce urea and ornithine. The ornithine returns to the liver for use in the urea cycle, while the urea is excreted[8]. The lack of the arginase enzyme results in excessive accumulation of nitrogen, in the form of ammonia (hyperammonemia) and arginine (hyperargininemia) in the blood. Untreated children may exhibit seizures, spasticity, short stature, and mental disability[9]. However, no systematic studies are available regarding the erythrocyte arginase activity and its association with plasma ammonia levels. Hence, the study was taken up to find out the association between plasma ammonia levels and arginase activity in erythrocytes.

Materials and Methods

Ethics

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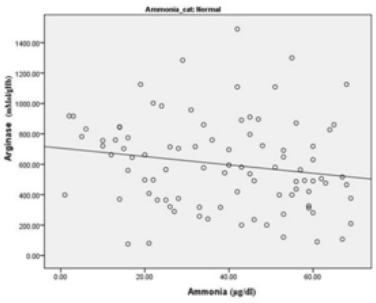

Figure 1: Correlation between ammonia and arginase activity in normal ammonia group. n=92, r=0.22, P=0.03.

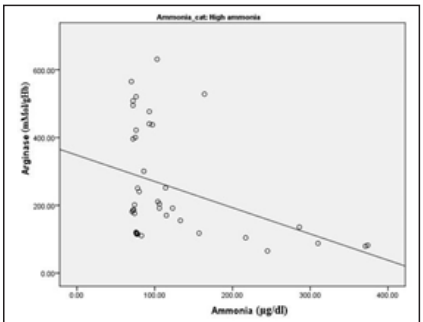

Figure 2: Correlation between ammonia and arginase activity in high ammonia group. n=41, r=0.39, P=0.01.

Ethical clearance certificate is obtained for the study following the an ethical number IEC 52/2017.

Study subjects

Study subjects included babies and children in the age group of 0–15 years recruited from Pediatrics Clinic, Kasturba Hospital, Manipal, Karnataka, India. The total numbers of samples analyzed are 133.

The specimen used

Erythrocytes separated from the whole blood sample collected with ethylenediamine tetraacetic acid (EDTA). Duration of the study was 6 months, from January 2017 to June 2017.

Inclusion Criteria

New borns and children upto the age of 15 years, whose blood samples were sent for ammonia estimation to biochemistry laboratory, from the Pediatrics Clinic, Kasturba Hospital.

Estimation of Arginase Activity

Arginase was estimated by taking arginine as the substrate and measuring the product ornithine colorimetricallyby its reaction with ninhydrin[10]. Blood collected in EDTA tube was centrifuged at 2000g at 4°C to extract red cells. The red cell extract was washed 3 times(2000g, 15 min) with 5 volumes of normal saline and diluted with 5mmol/L Tris buffer, pH 7.5. The suspension obtained was used for estimation of arginase activity and hemoglobin concentration[11]. Hemoglobin was estimated using Drabkin's method. Arginase activity is expressed as the amount in millimoles (mMol) of ornithine released per minute per gram hemoglobin under the assay conditions(Units/g hemoglobin). Ammonia was estimated by an enzymatic spectrophotometric method in Roche COBAS 6000 analyzer.

Statistic alanalysis

Statistical methods used are Mann–Whitney U-test for descriptive statistics and Spearman's Correlation analysis using SPSS 16.0.

Result

Based on the ammonia levels the samples are divided into two groups, namely, Group 1 with normal ammonia levels and Group 2 with high ammonia levels. The reference value of ammonia in plasma is 0–70 µg/dl. Any value above 70 µg/dl is included in the high ammonia group and <70 µg/dl is included in the normal ammonia group. Results of the present study are given in Tables 1 and 2,Fig. 1 and 2. Table 1showsthat there is a significant decrease (P<0.001) in arginase activity in high ammonia group as compared to the normal ammonia group (Interquartile range Q1-117.84, Q3-411.16). Table 2 and Fig. 1 and 2 show that there is a significant negative correlation between arginase and ammonia in Group 1(r=0.22,P=0.03) and Group 2 (r=0.39,P=0.01).

Table 1: Erythrocyte arginase activity in the two groups

Groups	Arginase (mMol/gHb)median (Q1, Q3)
Normal ammonia group (n=92)	571.22 (375.37, 793.15)
High ammonia group (n=41)	*191.74 (117.84, 411.16)
*P-value is <0.001	

Table 2: Group-wise correlation analysis between arginase and ammonia

Groups	r	P
Normal ammonia group (n=92)	0.22	0.03*
High ammonia group (n=41)	0.39	0.01*
*P-value-significant		

Disucssion

The defect in any of the urea cycle enzymes leads to hyperammonemia.The deficiency of a terminal enzyme of the urea cycle, namely, arginase is found to cause elevated ammonia levels[11]. Increased plasma arginine levels along with increased urinary orotic acid and dibasic amino acids are the hallmark indicators of arginase deficiency[11, 12]. Deficiency of Arginase I typically presents as a progressive neurometabolic disorder with spastic paraplegia, developmental retardation, and seizures accompanied by hyperargininemia[13]. Mild hyperammonemia is also a characteristic feature of arginase deficiency. As per review of literature by Scagliaetal.,patients with arginase deficiency whowere treated at birth with protein restriction and essential amino acid supplementation remained asymptomatic.This suggests that chronically elevated levels of arginine directly contribute to the neuropathology in these cases. In symptomatic patients whose treatment was begun at diagnosis, there is ample evidence that effective therapy stops the progressive neurological degeneration[14]. The incidence of hyperargininemia from U.S. newborn screening (NBS) data shows a minimal incidence of 1:1.2 M (2017) [12]. However, in India, no documentation of incidence of arginase deficiency, in particular, is available except for few case studies reported[11]. Even the NBS program conducted in different parts of the country did not include arginase in their panel. Hence, there is a need to include arginase assay in the routine panel of inborn errors of metabolism screening. Very few reports are available regarding the measurement of erythrocyte arginase activity in children. Recently diagnosis of arginase deficiency is more focused at the molecularlevel[15] or utilizing high-end technologies like tandem mass spectrometry[12]. In the present study, we have used simple, reliable, and

cost-effective method to measure the arginase activity which can be employed in most of the laboratory setups. The aim of the present study is to screen the babies with hyperammonemia for possible arginase deficiency. Erythrocytes were taken as the source of arginase which serves as an alternative to hepatic tissue. Arginase activity was also measured in the blood samples from children with normal ammonia levels and was taken for comparison. The significant decrease in the arginase activity in the high ammonia group (median= 191.7mmol/g Hb) as compared to the normal group(median=571.2mmol/g Hb) suggests that arginase deficiency could be the cause for hyperammonemia (n=41) in at least 50% of cases studied. These findings are further strengthened by the significant negative correlation observed between the plasma ammonia and erythrocyte arginase activity. Arginase deficiency meets the traditional criteria forNBS (Wilson and Jugner, 1968), with respect to prevalence and better treatment outcome; hence, arginase deficiency should be included in the NBS program. Results of the present study advocate the necessity of NBSfor arginase deficiency. The erythrocyte arginase activity reflects the hepatic arginase activity,and it is convenient to use erythrocytes for the estimation[2]. Hence, we conclude that this method can be used as a screening procedure to detect arginase deficiency in newborns, infants, and children using the limited volume of blood sample. Use of the erythrocytes as the source of arginase enzyme also avoids undue liver biopsy as a part of the diagnostic practice which offers an additional advantage.

Limitations of the Study

The study subjects belong to a wide age group (0–15 years). Dividing the subjects with narrow age range could make the study more specific.

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