Serum Cytosolic β-Glucosidase Levels In Neonatal Necrotizing Enterocolitis

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Abstract

Objective: This study was conducted to compare serum Cytosolic -Glucosidase (CBG) levels of age-matched control patients with those of infants with neonatal necrotizing enterocolitis (NEC), to determine eventual association between Serum Cytosolic -Glucosidase levels with intensity of the disease in NEC infants.

Methods: 82 neonates were divided into controls (group I, n=41), feeding intolerance (group II, n=15), and NEC (group III, n=26). Serum Cytosolic -Glucosidase was measured at the onset of feeding intolerance or NEC and at weeks 2–3 in control infants (Group I) by ELISA. Data were analyzed using descriptive statistics, non-parametric tests and Student t-test.

Findings: Median birth weights in three groups were 1761.3, 1951.9, 1893.7 g, median gestational ages were 33.6, 35.0 and 34.5 weeks and ages of sampling were 15.3, 14.7 and 15.1 days, respectively. The differences between NEC group, feeding intolerance group and the control group were not statistically significant ($P>0.05$). There was a trend toward an increase in Serum CBG levels (group I 36.5 nmmol/L), group II (112.4 nmmol/L) vs. group III (693.0 nmmol/L), (x^2=43.296, $P<0.01$). Infants in group III had highest CBG levels, compared with group II and I.

Conclusion: Serum CBG is elevated in NEC, maybe NEC is the reason of high level of Serum CBG. In addition, the serum CBG may have utility as an early marker of ischemia in patients at risk for NEC in future studies.

Introduction

Necrotizing enterocolitis (NEC) is an acquired gastrointestinal disease which primarily affects premature infants. It is the most common gastrointestinal emergency in the newborn and may lead to death in severely affected infants[1-3]. The incidence is 1–3 per 1,000 live births, occurring in 10% of infants born under 1500 grams and representing 2–5% of NICU admissions. Mortality has been reported to range from 9–28%[4-7]. The exact etiology and pathogenesis of NEC still remains unknown[8]. Although Bell et al[9] published staging criteria for NEC more than 20 years ago, currently, the diagnosis of NEC is based on clinical and radiographic findings. Once patients are diagnosed with definitive NEC (Bell's stage 2), significant intestinal damage has likely occurred. Accordingly, It is possible that earlier detection of

intestinal injury and institution of appropriate treatment might prevent the progression of the disease[10-11]. Several serum markers have been proposed as screening tests for intestinal ischemia such as IL-1ra, IL-6, C-reactive protein, and so on. These markers suffer from both poor sensitivity and specificity and are elevated only in severe NEC[12-14]. CBG is an enzyme found in intestinal epithelial cells, its specific activity in the combined intestinal compartments (duodenum, jejunum, ileum, and cecum) is approximately 6,000 U/mg, contributing to more than 80% of the total organ expression of CBG. Several studies suggest that serum CBG is elevated before transmural intestinal injury in the animal model occurs[15-16].

The aim of this study was to compare serum CBG levels of age-matched control patients with those of patients with NEC and to determine whether there was a significant difference.

Subjects and Methods

Three groups of neonates who were treated in the Neonatal Intensive Care Unit (NICU) at Quanzhou Children's Hospital, Quanzhou, China between May 2008 and June 2010 were enrolled in a prospective, ethically approved observational cohort study. The study groups consisted of neonates with a history of feeding intolerance or NEC as per Bell's staging II and III and who had long-term follow-up data available. A control group consisted of corrected gestational age- and weight-matched patients who were admitted to NICU without NEC, sepsis or septic shock, systemic inflammatory response syndrome, or an inborn error of metabolism. Infants with congenital anomalies or who had recent surgery (<1 week) were excluded from the study. There were three groups of infants studied. Controls (Group I) were defined as premature infants (37 completed weeks) admitted to NICU without any evidence of sepsis or GI pathology. Feeding intolerance (Group II) was defined as persistent gastric aspirates >50% of the feed volume with or without abdominal distension in the absence of culture-proven sepsis or radiological evidence of

NEC (Bell's stage I). Group III included infants with proven NEC (Bell's stage II and III). Data related to antenatal, perinatal and postnatal period were collected including birth weight, gestational age and Apgar score. Serum CBG level was determined within 24 h of onset of symptoms in the subjects in group II or group III and concomitantly in Group I.

Blood was collected by venepuncture and the serum harvested after centrifugation was stored at -70°C. Serum CBG concentration was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA). Briefly, samples were diluted 2 times and incubated on a precoated ELISA plate for 1 hour at room temperature. The samples were discarded, the ELISA wells were washed 3 times, and a biotinylated anti- -glucosidase was added for 1 hour. After further wash steps, the wells were incubated with streptavidin-HRP solution for 1 hour. After a final 3-wash steps, Substrate A and Substrate B were added to each well. Then Incubated for 10 min at 37 before the reaction was stopped by addition of H_2SO_4. The plate was read at 450 nm, and serum CBG concentration was quantified by comparison with a set of predetermined standards.

SigmaStat for Windows software (SPSS 13.0.) was used for statistical analysis. Data were analyzed using descriptive statistics. We performed a Chi Square analysis and used Student t-test, and Kruskal-Wallis test where appropriate. All non-normality data were expressed as range±SD (standard deviation). Statistical significance for all tests was defined as $P<0.05$.

Findings

Eighty-two subjects were identified who reached inclusion criteria for this study; there were 45 male and 37 female infants. Study group encompassed forty-one premature infants in group I, 26 infants in group II and 15 infants in group III. Demographic characteristics of the study groups are shown in Table 1. Median birth weights (mean±SE) in groups I, II and III in the

Table 1: Characteristics of patients according to study group

Parameter	Group I (n=41)	Group II (n=26)	Group III (n=15)
Sex (Male/Female)	8:7	18:8*	19:22*
Corrected gestational age (wk) [mean (SE)]	34.5±1.9	35 ± 2.1*	33.6±2.3*
Age of sampling (days) [mean (SE)]	15.1±2.6	14.7 ± 2.3*	15.3±2.8*
Birth weight (g) (mean±SE)	1893.7 ± 315.7	1951.9 ± 428.6*	1761.3±319.4*
Serum CBG level (nmmol/L) [mean (SE)]	36.5 ± 28.3	112.4 ± 108.5	693.0±211.6

* x^2=3.384, *P*>0.05 between group III and group II vs. group I.

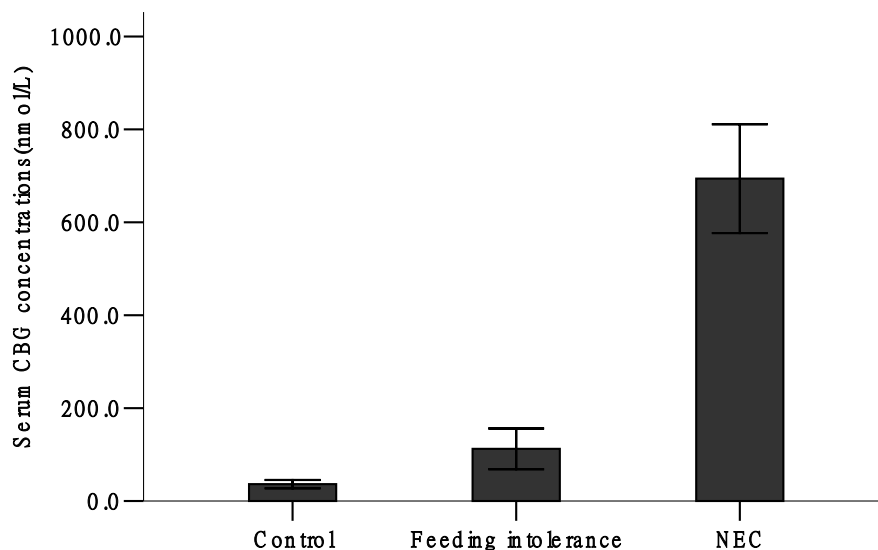x^2=43.296, *P*<0.01 between group III and group II vs. group I.

SE: Standard error

original study were 1761.3±319.4g, 1951.9± 428.6g, 1893.7±315.7g, median gestational ages (mean±SE) were 33.6±2.3 weeks, 35±2.1 weeks and 34.5±1.9 weeks and age of sampling were 15.3±2.8, 14.7±2.3, and 15.1±2.6 days respectively. The differences between Group III vs. Group I and II for gestational age and birth weight were not statistically significant (Table 1).

Serum Cytosolic -Glucosidase levels were measured in all 82 infants. However, The Serum CBG levels in groups I, II and III were 36.5±28.3 nmmol/L, 112.4±108.5 nmmol/L, 693.0±211.6 nmmol/L (Table 1). There was a trend toward an increase in serum CBG levels. Infants in group III had highest CBG levels compared with group II and I using Kruskal-Wallis test (Fig. 1).

Discussion

CBG, an enzyme found in intestinal epithelial cells, was elevated in adult guinea pigs in early ischemic and closed-loop occlusion intestinal injury[15]. Although CBG was present in several organs, the small intestine had by far the greatest activity. Reed et al found that elevated serum CBG activity preceded microscopic evidence of severe intestinal injury. In the animal model of NEC this elevation would occur before microscopic evidence of severe intestinal injury when the damage could be reversible. This study suggests that serum CBG may have utility as an early marker of ischemia in patients at risk for NEC, but currently no similar test is available for either

Fig.1: Serum CBG concentrations among NEC, feeding intolerance and controls

adult or neonatal intestinal injury. In addtion, the mechanism of naturally occurring human NEC is more complex [17,18]. So it is important to find whether elevated serum CBG activity would occur in human NEC.

In our study, there was a trend toward an increase in serum CBG levels. Infants in feeding intolerance group had higher CBG levels, compared with control group. NEC group had highest CBG levels. Our study suggests that elevated serum CBG levels can occur in early NEC (Bell's stage 1). Serum CBG levels were associated with extensive disease in infants with NEC. This finding is in agreement with previous reports describing the rapid rise of serum CBG after ischemic injury[19,20]. In addition, Morris et al[15] found a rapid rate of rise of serum CBG in animals undergoing intestinal ischemia and concluded that other organs expressing CBG did not contribute significantly to serum CBG activity. They demonstrated that CBG specific activity in the combined intestinal compartments was approximately 6,000U/mg, contributing to more than 80% of the total organ expression of CBG. Thus, in this animal model of NEC, the elevation in serum CBG likely resulted from intestinal injury.

There are several limitations to our study. Blood samples were taken only once. The study is a descriptive study, so we can only claim that NEC is the probable reason for high level of serum CBG. In future studies we could determine whether serum CBG has utility as an early marker of ischemia in patients with NEC. And it also should be aimed to find the normal levels of serum CBG in neonates and adults to assist clinicians in the diagnosis of NEC allowing earlier therapeutic intervention with improved survival rates.

Conclusion

In conclusion, this study confirms that serum CBG is elevated in NEC, maybe NEC is the reason of high level of Serum CBG. In addition, serum CBG may have utility as an early marker of ischemia in patients at risk for NEC in future studies.

Acknowledgment

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