Diagnosis and clinical observation of lactose-free milk powder on treatment of neonatal diarrhea

Jingyan Liu*, Jing Chang, Aimei Yao, Yulian Hu, Yuxiao Yuan, Fengqin Yu, Zhanmin Ma, Guangzhou Wang and Xiang Zhao
Women & Infants Hospital of Zhengzhou, Zhengzhou, Henan Province, P.R. China

Abstract: Neonatal lactose intolerance syndrome is a series of digestive system symptoms caused by the lack of lactase, and could not fully digest the lactose in breast milk or cow milk. Lactose is one of the disaccharides mainly existed in mammalian milk. Lactose content in breast milk is 7.2g/100ml, cow milk is 4.7g/100ml. Dairy products are the main energy sources for the newborn, and lactose provides 20% energy for infants. During the growth of the newborn, lactose not only play an significant role in energy supply, but also involve in the development of the brain growing. This study mainly studied the lactose development features, the reasons for lactose intolerance, and the measures to treat lactose deficiency.

Keywords: Lactose-free milk, low lactose formula milk, neonatal diarrhea, diagnosis.

INTRODUCTION

Lactose is mainly absorbed in jejunum and ileum. Under the secretion of small intestinal epithelial cells, the lactose is hydrolyzed into glucose and galactose and absorbed by cell active transportation. Lactose enzyme is also called as β-lactase-phlorizin-hydrolase, or LPH, of which the activity increase gradually from proximal to distal segment of the duodenum, and reach the peak in jejunum or proximal of ileum, then reach the bottom level in terminal ileum. If any step of lactose absorption had problem, it would result in higher lactose concentration in the lumen, and cause the lactose indigestion (Brown-Esters *et al*., 2012). If dairy food go to colon, the intestine flora could disintegrate lactose into acidic material such as lactic acid and short chain fatty acids, and release hydrogen and methane, which lead to abdominal distension, while, could stimulate intestinal peristalsis hydrocarbon, which cause pneumatic diarrhea.

Lactose development characteristics of newborn

Lactose enzyme could be detected from gestational 12 weeks to gestational 34 weeks, and it gradually increased, and the lactose enzyme activity was equivalent to 30% of the full-term infant. The lactose enzyme development of full-term infant was basically mature (Montgomery *et al*., 1999). So the premature infant had poorer lactose tolerance than term infant. Under the function of intestinal epithelial cells brush border, enzyme became lactose enzyme and be decomposed into galactose and glucose. 50% galactose could further metabolize in the liver cells after the absorption of intestinal epithelial cells. Under the conversion of galactolinase, galactose-1-phosphate uridyl transferase and uridine diphosphate galactose epimerase, and the galactose generate glucose-1-phosphate entered gycolysis pathway and released energy. Lactose could be the raw material for the synthesis of galactoside and compose cerebroside content, which closely related to the rapid growth of infants after born. Lactose enzyme mainly expressed in the small intestinal villus. Compared with other enzymes, mucosal was more vulnerable to the impairment. In disaccharidase, lactase enzyme was the first enzyme affected by the intestinal mucosa impairment, and the final enzyme for recovery. Lactose without digestion in small intestine could enter the colon and became short-chain fatty acid by glycolysis. Some scholar supposed that the glycolysis in colon of carbohydrate without digestion might related to the incidence of necrotizing enterocolitis, also named NEC. While, some scholars held that the carbonhydrates in breast milk was mainly lactose, with higher lactose content. The NEC incidence rate was not high might due to the intestinal protective factors of breast milk, instead of the lactose content.

Mention reference

Encoding genes of the lactasegene (LCT) located on the long arm of chromosome 2q21-22, length was about 50kb (Itan *et al*., 2010). When gestational age was around 8 weeks, the lactose enzyme in epithelial cells of the small intestine begin to express. The expression of lactose enzyme reached the peak level since the 34 gestational weeks till born, and slowly decreased months after born. It was considered that the single nucleotide polymorphisms of LCT upstream of C-13910T could regulate the expression of lactose enzyme. Genotype CC was associated with low lactose disease, while genotype CT and TT was associated with continuous expression of lactose. But the gene mutation did not responsible for all the low lactose enzyme disease. Enattah researched that mutation G-22018A involved in regulation of lactose enzyme expression in the 236 cases of patients with low

**Corresponding author:* e-mail: zmabc2015@sina.com

lactose enzyme of nine family (Enattah *et al*., 2002). While, these two mutation could not explain all the low lactose disease. Related research demonstrated that it was not significant of the correlation between these two mutations and lactose enzyme expression of the people in South Africa, the Central Asia, the Middle East and the Southeastern Europe. Lactose deficiency was related to race, genetic, geographical environment and other factors. The incidence of black people was far higher than white people, Asian people was 90~100%. Investigation showed that the average incidence rate of lactose malabsorption was 87.8% for Chinese children aging 11 to 13 years old. The incidence of lactose intolerance was 30.5%.

Reason for lactose intorlerance

When the amount of lactose in the small intestine exceeded the digestive ability of lactose enzyme, the lactose intolerance could happen. The main reason were as followings.

Congenital lactose deficiency

The low lactose activity or deficiency after born was mostly due to the autosomal recessive inheritance, which was relatively rare. There was another phenomenon called instant onset in neonatal period, infants usually developed the disease after the taken of breast milk, due to the non adaption to breast milk. After taken the breast milk, infants usually had obvious vomiting, watery diarrhea, increased faeces acid and accompanied by symptoms, such as abdominal distension and the bowel sounds hyperfunction. After the stop of breast milk feeding, the above symptoms disappeared quickly. If the lactose free formula milk was not taken, the infants would have life risk.

Primary lactose deficiency

The primary lactose deficiency was the most common cause of lactose intolerance, also called adult-typehypolactasia biopsy. For these patients, most cases had normal lactose expression after born. The lactose activity gradually declined during the growing up. The lactose enzyme activity of small intestine epithelial of mammals included human adult decreased to 5~10% of the level at birth, which may be associated with reduced enzyme lactose gene expression (Ridefelt and Hakansson, 2005). The pathogenesis of this symptom could happen in the neonatal period. Lactose matured latest among all dosaccharidase, and lowest content. The intestine mucous membrane of neonates, especially the premature infants, was not mature, and had poor digestion and absorption lactose ability. Therefore, neonates, especially premature infants could develop primary lactose enzyme deficiency. Primary lactose deficiency occupied 70% of the world's population, and was the most common cause for lactose intolerance and lactose malabsorption. The incidence of different ethnic groups were very different, Asian people had the highest level were nearly 100%, followed by Jews, the South Africans, Black race people, the rate was 50~80%; The lowest incidence of white people is 2~15% (Ozdemir *et al*., 2009). As not all primary lactose deficiency could have clinical symptom, food for adults should mainly be the food without sufficient lactose. The less intake of lactose could not cause clinical symptom.

Secondary lactose deficiency

The secondary lactose deficiency was common in neonates, which was most caused by infectious diarrhea, malnutrition, chronic inflammation of intestinal mucosa, systemic infection, anoxia, gastrointestinal surgery, decreased or lost of lactose enzyme secretory epithelial cells, and declined lactose enzyme secretion. The rotavirus infection often impaired the intestine mucosa villus epithelial cells, and reduced the intestinal disaccharidase, especially lactose enzyme activity, and caused secondary lactose intolerance, and happened permeability diarrhea. With the recovery of intestinal mocasal epithelial cells impair, lactose enzyme activity could improve as well.

Clinical manifestation of lactose deficiency

It was common for the lactose enzyme deficiency for newborn babies, not all could have clinical symptom (Neu, 2007; Morales *et al*., 2011). Some infants only had the reduced enzyme lactose expression or declined activity. It was also called lactose malabsorption due to the less lactose intake and without clinical symptom. According to the domestic research, the incidence of neonatal lactose malabsorption was around 40%, and occupying 12~30% of all patients with lactose deficiency (Zheng and Yu, 2003). If the deficiency of lactose lead to malabsorption and cause diarrhea, vomiting, abdominal distension, abdominal pain and other clinical symptom, which was known as lactose intolerance. The character was with digestive tract symptom after intake of lactose, and the severity of the symptoms was associated with the amount of lactose intake. When the lactose enzyme activity reduced or deficient, a host of lactose remained in the lumen. Due to the permeation, the water and small molecular substances of cells enter lumen, thus fluid volume increased, and caused the watery stool. The undigested lactose that entered the colon with bowel movement could be catabolized by intestinal flora and produced lactic acid, acetic acid and hydrogen gas, which resulted in abdominal distension, abdominal pain, increased exhaust, and exacerbate the symptom (Cao, 2011). At the same time, the catabolized lactic acid, short chain fatty acid made the change of intestinal ph value, and acidic stool. The lactose intolerance caused diarrhea stool was yellow or yellowish green, egg drop soup or thin paste, with milk block and bubbles and stink smell. Diarrhea could be up to or more than 10 times per day, the serious person could appear mucous bloody stool and small intestine necrosis. Lactose intolerance was the cause of diarrhea, which caused the impair of intestinal mucosa cells, further reduced lactose secretion, causing vicous

cycle and aggravating illness. In addition, due to long time lactose intolerance and vomiting, the physical development affected patients with lactose intolerance. Compared to normal infants, the weight would be less and height would be shorter, and more incidence rate of anemia and osteoporosis and other disease (Yue *et al*., 2010).

In addition, breastfeeding infants often have diarrhea as well, who were usually diagnosis as physiological diarrhea. But clinical finding also proofed that the diarrhea could significantly reduced once the babies were feed by formula milk. Domestic scholars supposed that physiological diarrhea might be associated with lactose intolerance (Yao *et al*., 1991). In this paper, as the lactose content of breast milk was higher than formula milk, the reason why incidence rate for patients with breast milk intake was higher than patients with formula milk intake might related to lactose intolerance, which required to be confirmed by rigorous clinical research.

Lactose deficiency of premature infants and its feed tolerance

The development of small intestine lactose enzyme was affected by feeding method and food ingredients, Shulman researched the lactose enzyme activity for premature infants with early enteral feeding was greatly creased than premature infants with late enteral feeding, 10~28 days after born. The lactose enzyme activity for premature infants with breast milk feeding was higher than that with formula milk feeding 10 days after born. Lactose enzyme activity had inverse proportion with time of full enteral feeding and abdominal X-ray check-up frequency. Therefore, early feeding could increase the activity of lactose enzyme for premature infants. Lactose enzyme activity was an indicator of the maturity of the intestinal development detection and cannot affect the clinical prognosis. With the increase of survival rate of premature infants, feeding intolerance of preterm infants became an important factor to affect the premature development and disease recovery. In the cause of premature infant feeding intolerance, the core factors were lactose low activity and lactose intolerance. In 1990s, Shulman started to study the premature infants' lactose digestion ability of formula milk. In the three formula of pure lactose, polymer of lactose and glucose, and pure glucose polymer, premature infants had poorest absorption ability on pure lactose formula milk, while, it had better absorption ability on polymer of lactose and glucose and pure glucose polymer, of which the absorption ability was not related to postnatal age and feeding history. The lactose absorption speed for pure formula milk for pre mature infants was faster than mixed feeding of breast milk and formula milk.

Common diagnostic method for lactose enzyme deficiency

There were many diagnosis methods for lactose enzyme deficiency. Most were only used for research, and rarely used in clinical field. The diagnosis for newborn was less. The diagnosis methods of lactose intolerance were mainly below.

Hydrogen breath test

Under normal circumstances, human body did not produce hydrogen gas. The undigested lactose catabolized by bacteria in colon could produce hydrogen, and be absorbed into blood, and then be discharged through inhale and exhale. The detected level of exhaled hydrogen could indirectly reflect the condition of lactose digestion and absorption. Method was to detect the hydrogen concentration 3 hours after the intake of lactose for 1~ 2g/kg, fasting level increased more than 0.02mmol/L and was judged as lactose malabsorption. This method was the most reliable detection for adult diagnosis of lactose intolerance, which the document called as the golden standard. The method operation was without impairment, sensitive, accurate and simple, the only requirement was trace hydrogen tester. As the operating time was several hours, which need the good cooperation of subjects. Because the infants usually cry and have hyperventilation, sufficient fasting and poor ability of intestinal bacteria catabolizing lactose affected the results to be false negative. Therefore, this method was rarely used. But, there were some study report for newborn doing hydrogen breath test.

Excrement reductive sugar and ph level detection

The lactose undigested in intestine could be educed along with the excrement and urine. Stool was acidic, and could detect lactose catabolism condition through excrement reductive sugar and ph detection. The most applied detection of excrement reductive sugar was method of lead acetate plus ammonium hydroxide and modified Ban' reagent method. Positive was shown as reductive sugar ++. Excrement ph detection could be widely applied on strip detection, ph level less than 5.5 indicated the deficiency of lactose enzyme, which the advantage was noninvasive, simple, cheap, suitable for infants. And the disadvantage was the requirement of fresh specimens. As the reductive sugar of stool was mainly in stool liquid, the stool liquid should not stain on the diaper when it been collected. It made the difficult of specimen collection. It also easily affected by the intestine bacteria environment. The interpretation results for samples may had error due to the specimen color.

Lactose tolerance test

The fasting glucose level should be measured. Then the certain quantity of lactose (50g for adults, 1~1.5g/kg) should be taken as the loading quantity. The glucose concentration would be detected after half an hour. What suggests the lactose malabsorption was that the increased blood sugar less than 20mg/dl and with clinical symptom (Shulman *et al*., 1998; Shulman *et al*., 1995). Although

the test was invasive examination, it could be used to measure peripheral blood sugar, and could be used in the detection of lactose enzyme deficiency for infants. This method could be impacted by gastric emptying, intestine peristalsis, nutritional status and glucose metabolism. The sensitive was 77~96% and the specific degree was 76~96%.

Urine galactose detection

The principle was urine galactose detection after taking loaded lactose. Infants shall evacuate urine, then take breast milk or lactose-contented milk, and then the urine should be collected, purified, and compared with galactose standard liquid on the reaction device. There were some galactose oxidise remained in the reaction device. The galactose could generate hydrogen peroxide and glocodialohexose. Under the assist of 4-aminoantipyrine, 3,4-dichloro-2-hydroxybenzenefulfonic acid, it was oxidated to be red color, to some degree, the red color depth was proportional to the concentration of galactose. In recent years, the clinical study on oxidase detection of urine galactose was widely applied. Nearly five years, the domestic scholars respectively did the urine galactose detection for 160 cases of infants with diarrhea. The results demonstrated that β-lactam antibiotics could cause drug-induced secondary lactose intolerance. The detection of urine galactose intolerance for infants and young children were widely employed abroad, not been used for clinical purpose in domestic medical field. Its advantage was noninvasive, convenient, accurate, suitable for the diagnosis of neonatal lactose deficiency.

Gene detection

There was an new findings of a gene mutation in northern European. The cytosine (C-13910T) was replaced by thymine in the upstream of lactose enzyme coding gene, which enabled the lactose enzyme be expressed in the whole life of this people. Polymerase chain reaction method could be utilized to screen the already discovered single nucleotide polymor-phism G-22018A and C-13910T for the early diagnosis of the lactose deficiency. Because the two genetic mutation could not explain all the lactose intolerance, and there were still undiscovered mutations of genetic mechanism. Therefore, the accuracy was not high, and it was used for genetic research, which was not widely applied in clinical field.

Lactose rapid detection

Lactose rapid detection was the relatively new diagnostic method in recent years, namely, the lactose rapid detection of the small intestinal epithelium mucosa cells through endoscopic device. This method had high accuracy. As it was an invasive procedures for newborn babies and more difficult to operate, which was not easily accepted by parents.

The treatment of lactose intolerance

Dietary therapy

Dietary avoid was the diagnosis method of lactose intolerance, also the main treatment of lactose intolerance. Based on the clinical symptom severity, it was required to choose the lactose-free formula milk or low lactose formula milk. In the lactose-free formula milk, lactose was usually replaced by maltodextrin and corn flour. But the application course of treatment normally should not be more than 2 weeks due to its low heat. The clinical symptom of primary lactose intolerant patients was closely related to the amount of lactose intake. For neonatal with severe lactose intolerance, it should choose lactose-free formula milk, the symptom could relieve in 1~2 days. Then the low lactose formula milk should be taken, and take diary products in a repeated small amounts, in order to enhance intestine lactose tolerance. When the lactose tolerance was enhanced, it should gradually return into breast milk or formula.

As the lactose content in breast milk was higher than formula milk, it was still controversial whether the breast milk should be replaced by lactose-free formula milk or normal preterm infant formula for very low birth weight infants (VLBWI). Although the lactose enzyme activity of VLBWI was low, and with poor lactose tolerance. As the VLBWI had rare month intake after birth, the intake quantity of lactose was relative limited, thus could not cause lactose intolerance.

In 1999, Griffin had studied the tolerance of low lactose formula and standard preterm formula milk for preterm infants patients (Griffin *et al*., 1999). The two groups of preterm infants patients (more than 36 weeks of gestational age, birth weight less than 1800g) was respectively taken low lactose) lactose was replaced by maltose and 1% of lactose to carbohydrates and standard premature infant formula (lactose energy was 24kal per ounces), and compare the tolerance of feeding. The results demonstrated that the intestine heat calorie intake of low lactose formula group improved better than lactose-content formula group, and had faster body weight growth, reduced gastric retention, low incidence rate of stopping feeding and shorter time of non-mouth-feeding. The incidence rate of NEC from the two groups had no difference, which indicated the early feeding help to increase the lactose enzyme activity. There were other scholars researched that the lactose enzyme activity was higher for neonatal who started feeding 4 days after birth than that of 15 days after birth. The lactose enzyme activity of pure breast milk was still higher than that of pure formula milk. Although the preterm infants had low enzyme activity, it could be induced. As the lactose enzyme activity of full-term infants was poorer than pre-term infants, it not suggested to take lactose enzyme for preterm infants to increase the absorption ability of lactose. Erasmus researched to feed the lactose-added

preterm formula milk to preterm infant patients (Erasmus *et al*., 2002). The results showed that the level of weight increase and serum albumin was higher than control group. But there was no effect on height, head circumference, tolerance and NEC.

Many infant formula manufactures had realized that the poor tolerance of neonatal especially the preterm infants. Therefore, the increase of lactose content was replaced by glucose polymer in preterm infants formula milk, in order to improve the absorption ability. However, preterm infants still could not tolerate the small amount of lactose in standard preterm formula milk.

With the understanding of lactose intolerance, varies of lactose-free formula milk come to birth. It was controversial if these lactose-free formula was total lactose-free, and these lactose-free formula milk could be used for infants with galactosemia (Morlock *et al*., 2014).

Probiotics

Individual with lactose intolerance could tolerate yogurt and other fermented daily products very well. The intake activated yogurt products could improve the lactose digestion of patients with lactose enzyme deficiency. Due to the function of β-galactosidase in activated yogurt, the 25~50% lactose in milk could be catabolized by bacterium acidi lactici, and increased the lactose content in yogurt. Therefore, the low lactose yogurt was easy to be digested and absorbed for patients suffering lactose intolerance. Many probiotics was related to lactose enzyme. As bacterium acidi lactici could generate lactose enzyme, and delayed the gastric emptying rate, and extended the intestinal transit time. Bifidobacterium and lactobacilus could catabolize lactose and generate acid, instead of gas in the catabolism of lactose, without increasing the osmotic pressure. At the same time, the absorption of intestine short chain fatty acids should be strengthened, and was beneficial to alleviate the symptoms of lactose intolerance. Chen reported that it had gained good effect for the utilization of bilidobacterium and clostridium butyricum treatment on infants with secondary lactose intolerance under the circumstance of non-stop breast milk feeding (Chen, 2007).

Added lactose

The most significant pathological change of lactose intolerance syndrome was lactose enzyme deficiency or low activity. Theoretically, lactose supplements was the best choice. If lactose enzyme was added into milk, the 70 ~ 80% lactose could be hydrolyzed. The effect of lactose enzyme was related to the lactose amount, lactose enzyme amount and the enzyme activity remained in gastrointestinal tract. The best ph level for lactose enzyme function was 5.5~6.0. It was usually destroyed by stomach acid and affected the curative effect. Furthermore, the high manufacturing cost and the high price, it was not been widely applied in China, although it had already been applied abroad. Some domestic hospital had started to apply lactose enzyme on the treatment of infant with acute diarrhea accompanied with secondary lactose intolerance. It would gain a better treatment effect give the lactose enzyme along with the milk feeding. The advantage of this medicine was to continue the original feeding method without stopping breast milk feeding, and improve the symptoms of diarrhea (Sun *et al*., 2011; Lu *et al*., 2013).

CONCLUSION

So sum up, although there was many diagnostic method for lactose intolerance, few were suitable for neonatal. The neonatal diarrhea was the normal disease, of which the lactose intolerance occupying a larger proportion. Therefore, it was necessary to explore a suitable method for neonatal diagnosis method of lactose intolerance. The treatment of neonatal lactose intolerance was mainly to apply lactose-free milk, and supplemented by probiotics. The application of oral lactose started late in domestic field, of which the effect evaluation required to possess a large quantity of sample for prospective clinical study.

REFERENCES

Brown-Esters O, McNamara P and Savaiano D (2012). Dietary and biological factors influencing lactose intolerance. *IDJ*, **22**: 98-103.

Cao BX (2011). Analysis on lactose intolerance for abdominal distension neonatal. *JPM*, **27**: 986-987.

Chen Q 2007). The combined therapy of bifidobacterium and clostridium butyricum on treatment of 72 cases of infants with secondary lactose intolerance. *TMJ*, **35**: 237.

Enattah NS, Sahi T and Savilahti E *et al* (2002). Identification of a variant associated with adult-type hypolactasia. *Nat. Genet*, **30**: 233-237.

Erasmus HD, Ludwig- Auser HM and Paterson PG *et al* (2002). Enhanced weight gain in preterm infants receiving lactase- treated feeds: A randomized, double-blind, controlled trial. *J. Pediatr.*, **141**: 532-537.

Griffin MP and Hansen JW (1999). Can the elimination of lactose from formula improve feeding tolerance in premature infants? *J. Pediatr.*, **135**: 587-592.

Itan Y, Jones BL and Ingram CJ *et al* (2010). A worldwide correlation of lactase persistence phenotype and genotype. *BMC. Evol. Biol.*, **10**: 36.

Lu R, Diao ZY and Xu XX (2013). Clinical observation of treatment of infants and young children with acute diarrhea diarrhea and secondary lactose intolerance. *JPP*, **19**: 23-25.

Montgomery RK, Mulberg AE and Grand RJ (1999). Development of the human gastrointestinal tract: twenty years of progress. *Gastroenterology*, **116**: 702-731.

Morales E, Azocar L and Maul X *et al* (2011). The European lactase persistence genotype determines the lactase persistence state and correlates with gastrointestinal symptoms in the Hispanic and Amerindian Chilean population: A case-control and population-based study. *BMJ. Open,* **1**: e000125.

Morlock GE, Morlock LP and Lemo C (2014). Streamlined analysis of lactose-free dairy products. *J. Chromatogr. A.*, **1324**: 215-223.

Neu J (2007). Gastrointestinal maturation and implications for infant feeding. *Early Hum Dev.*, **83**: 767-775.

Ozdemir O, Mete E and Catal F *et al* (2009). Food intolerances and eosinophilic esophagitis in childhood. *Dig. Dis. Sci.*, **54**: 8-14.

Ridefelt P and Hakansson LD (2005). Lactose intolerance: Lactose tolerance test versus genotyping. *Scand J. Gastroenterol.*, **40**: 822-826.

Shulman RJ, Feste A and Ou C (1995). Absorption of lactose, glucose polymers, or combination in premature infants. *J. Peduatr.*, **127**: 626-631.

Shulman RJ, Schanler RJ and Lau C *et al* (1998). Early feeding, feeding tolerance and lactase activity in preterm infants. *J. Pediatr.*, **133**: 645-649.

Sun XH, Ren LH and Dai HS (2011). Application of Lactose intervention on treatment of rotavirus enteritis combined secondary lactose intolerance syndrome. *MI*, **6**: 2446-2447.

Yao FB, Wang JK and Shi WS (1991). Physiological diarrhea and a special type of lactose intolerance: 60 cases analysis. *JCP*, **9**: 149-150.

Yue YL, Cheng F and Yu F *et al* (2010). Analysis of lactose malabsorption and trace element detection for infants with lactose intolerance. *CJLD*, **14**: 1653-1654.

Zheng Z and Yu RJ (2003). Screening and discussion on early neonatal lactose malabsorption and lactose intolerance. *JN*, **18**: 244-246.