Breast milk β-glucuronidase levels in hyperbilirubinemia

Şule Yiğit[1], Gönenç Ciliv[2], Canan Aygün[1], Gülşen Erdem[1]

[1]Department of Pediatrics, and [2]Department of Biochemistry, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Yiğit Ş, Ciliv G, Aygün C, Erdem G. Breast milk β-glucuronidase levels in hyperbilirubinemia Turk J Pediatr 2001; 43: 118-120.

Breast milk β-glucuronidase was thought to be one of the etiological factors in the pathogenesis of late-onset breast-milk jaundice, but results of these studies are conflicting. In this study breast milk β-glucuronidase levels were determined in groups with physiologic jaundice, early breast-feeding jaundice and late breast-milk jaundice. No difference in β-glucuronidase levels of these three groups was found in samples taken on the 4th and 15th days of life. β-glucuronidase activity in breast milk declined from the 4th to 15th day in all groups.

These results imply that factors other than breast milk β-glucuronidase activity should be investigated to reveal the pathogenesis of late-onset breast-milk jaundice.

Key words: β-glucuronidase, breast-milk jaundice, hyperbilirubinemia.

Neonatal jaundice associated with breast-feeding is a problem frequently encountered in neonatology[1]. Early breast-feeding jaundice occurs in the first days of life in breast-fed newborns, as effective breast-feeding is not established[2]. Late breast-milk jaundice is defined as jaundice occurring after the first three to five days of life when there is no hemolysis, and weight gain and intestinal function are normal[1,3]. Of infants who develop bilirubin levels high enough to require phototherapy and who do not have evidence of isoimmunization or other obvious hemolytic disease, 80 to 90 percent are fully or partially breast-fed[4]. Several etiological hypotheses have been postulated to explain the pathophysiological mechanisms of late breast-feeding jaundice. These hypothesis are related to substances such as pregnanediol and non-esterified fatty acids which are thought to inhibit glucuronyl transferase, and there are additional mechanisms-proposed responsible such as increased enterohepatic circulation of bilirubin and increased β-glucuronidase activity[5-15]. There are conflicting reports on β-glucuronidase activity in early and late breast-feeding jaundice[5,7,9-11,13-15]. Our previous report showed no effect of β-glucuronidase activity in early breast-feeding jaundice[7]. In this study we intended to compare maternal milk β-glucuronidase levels in babies with early and late breast-milk jaundice.

Material and Methods

The study group consisted of 41 healthy jaundiced newborn babies, which were exclusively breast-fed. The control group consisted of 18 healthy breast-fed newborn babies without visible jaundice. The study group was screened to exclude those with blood group incompatibility, hypothyroidism, cholestatic disorders, diabetic mothers, asphyxia, septicemia, cephalhematoma, bruising and glucose-6-phosphate dehydrogenase deficiency. Information on the perinatal period, birth weight, maternal drug use, first feeding time, feeding frequency, Apgar scores and postnatal weight loss was obtained for each infant. After informed consent was taken, two samples of breast milk were collected with a manual pump from the mothers. The first sample was collected between the 3rd-5th days; the second sample was collected on the 15th day. Bilirubin levels of the babies were determined at the same time. The samples were kept at -20 °C until analyzed.

The β-glucuronidase assay was performed using phenolphthalein mono β-glucuronic acid as substrate (Sigma kit No.325), and the results

were expressed as modified Sigma Units/ml. Statistical lanalysis was performed using Mann-Whitney U and chi-square tests.

Results

Around the 4th day of life 27 (65.8%) of the 41 infants in the study group had bilirubin peak levels between 7-12 mg/dl (physiologic jaundice) while the other 14 (34.2%) babies had bilirubin peak levels higher than 16 mg/dl (significant hyperbilirubinemia, breast-feeding jaundice)[1,16]. The infants with significant hyperbilirubinemia were treated with phototherapy according to our hospital practice. There was no difference between groups in first feeding time, feeding frequency, and weight loss within the first week of life. On the 15th day of life 22 (53.6%) of the 41 infants had bilirubin levels higher than 7 mg/dl (prolonged jaundice). Nine of these 22 infants were in the physiologic jaundice group and 13 were in the early breast-feeding jaundice group around the 4th day of life. These nine infants with prolonged jaundice were excluded from the physiologic jaundice group. Table I shows the characteristics of the three groups: those with physiologic jaundice, breast-feeding jaundice and the control group around 4th day of life. There was no significant difference in β-glucuronidase levels between the three groups around the 4th day of life. Furthermore, no correlation was found between bilirubin and β-glucuronidase levels in those with physiologic jaundice, or in the breast-feeding jaundice group ($r = -0.33$, $r = 0.42$). On the 15th day of life no significant difference was found in β-glucuronidase levels between the prolonged jaundice group and the infants with bilirubin levels below 7 mg/dl. There was also no sifnificant difference in β-glucuronidase levels between the prolonged jaundice group and the control group on the 15th day (Table II). In these groups no correlation was found between bilirubin and β-glucuronidase levels on day 15. In all groups there was a decrease in β-glucuronidase levels between the 3rd-5th and 15th days of life.

Table I. β-glucuronidase Levels Around the 4th Day of Life and Characteristics of Groups

	Physiologic jaundice group (n=18)	Breast-feeding jaundice group (n=14)	Control group (n=18)	P
Birth weight (g)	3330 ± 319 (2640-3900)	3315 ± 420 (2600-3900)	3341 ± 215 (3000-3750)	> 0.05
Gestational age (week)	38.8 ± 0.9 (37-40)	38.4 ± 1.0 (37-40)	39.2 ± 1 (37-41)	> 0.05
Maternal age (year)	29.1 ± 5.7 (22-40)	28.5 ± 6.3 (21-38)	28.6 ± 4.9 (20-36)	> 0.05
Serum bilirubin levels (mg/dl)	10.2 ± 1.7 (7.9-13.1)	18.2 ± 1.8 (16.2-21.8)	-	0.00
β-glucuronidase levels (U/ml)	385.6 ± 364.6 (144-1350)	259.9 ± 281.3 (60-1179)	276.0 ± 125.9 (84-621)	> 0.05

Table II. β-glucuronidase Levels on the 15th Day of Life and Characteristics of Groups

	Babies with bilirubin levels < 7 mg/dl (n=19)	Babies with bilirubin levels > 7 mg/dl (n=22)	Control group (n=18)	P
Birth weight (g)	3339 ± 312 (2640-3900)	3252 ± 396 (2600-3900)	3341 ± 215 (3000-3750)	> 0.05
Gestational age (week)	38.7 ± 0.8 (38-40)	38.6 ± 1.0 (37-41)	39.2 ± 1.1 (37-41)	> 0.05
Maternal age (year)	28.8 ± 5.7 (22-40)	29.4 ± 5.8 (21-39)	28.6 ± 4.9 (20-36)	> 0.05
Serum bilirubin levels (mg/dl) (day15)	3.6 ± 1.9 (0.7-7.0)	9.4 ± 1.7 (7.1-13.1)	-	0.000
β-glucuronidase levels (U/ml) (day 15)	104.2 ± 47.7 (45-222)	134.6 ± 78.3 (33-336)	128.1 ± 72.5 (42-294)	> 0.05

Discussion

Breast-feeding is a common cause of jaundice in normal newborns in the first week of life and beyond[1,16]. The cause of early-onset breast-feeding jaundice has been related to the frequency of feedings and to inadequate caloric intake[3]. Late-onset breast-milk jaundice is thought to be associated with an abnormality in the composition of breast milk[16]. Breast milk β-glucuronidase was thought to be an etiological factor in the pathogenesis of late-onset breast-milk jaundice, and studies were performed to clarify the effect of β-glucuronidase activity in human milk[5,7,9-11,13-15]. The results of these studies are conflicting. Gourley et al.[11] found that bilirubin levels were related to concentrations of β-glucuronidase in breast milk. However, other studies showed no correlation[5,7,9,13,15]. As breast-milk jaundice may show a broad clinical spectrum ranging from physiologic jaundice to significant hyperbilirubinemia[1], this study was undertaken to investigate the β-glucuronidase levels in breast milk in two groups of hyperbilirubinemic babies with physiologic jaundice and significant hyperbilirubinemia. In this study, similar to other studies[5,7,9,13,15], we did not find any correlation between bilirubin and β-glucuronidase levels in the prolonged jaundice group. Furthermore, the changes in β-glucuronidase concentration in breast milk were found to decrease from the 4th to the 15th day.

Babies with physiologic jaundice in this study were also investigated for breast milk beta-glucuronidase levels. The onset and time of peak bilirubin levels of physiologic jaundice and early-onset breast-milk jaundice are similar, but in early breast-feeding jaundice, peak levels exceed the normal range of physiologic jaundice on the 3rd or 4th day of life. Although no significant statistical difference was shown in β-glucuronidase concentrations between any of the groups (Table I), it is surprising to observe the highest mean breast milk β-glucuronidase level in the physiologic jaundice group.

In the prolonged hyperbilirubinemia group, 13 of 22 infants had been grouped as early breast-feeding jaundice around the 4th day of life. Although there is a classification as early-onset and late-onset breast-feeding jaundice[1,3], our results indicate that nearly all (except one) babies with early-onset breast-feeding jaundice had prolonged hyperbilirubinemia on day 15. These results show that distinction of early and late type breast-feeding jaundices may not be clear, as some cases may overlap.

These results confirm that a simple hypothesis, involving the single action of β-glucuronidase, is insufficient to explain the pathogenesis of breast-feeding jaundice.

REFERENCES

1. Hallmark LP, Stevenson DK. Neonatal jaundice and liver disease. In: Fanaroff AA, Martin RJ (eds). Neonatal-Perinatal Medicine. St Louis: Mosby, 1997: 1345-1389.
2. Carvalho MD, Klaus MH, Merkatz B. Frequency of breast-feeding and serum bilirubin concentration. Am J Dis Child 1982; 136: 737-738.
3. Lascari AD. Early breast feeding jaundice: clinical significance. J Pediatr 1986; 108: 156-158.
4. Maisels MJ, Gifford KL, Antle CE, Leib GR. Jaundice in the healthy newborn infant: a new approach to an old problem. Pediatrics 1988; 81: 505-511.
5. Alonso EM,m Whitington PF, Whitington SH, Rivard WA, Given G. Enterohepatic circulation of nonconjugated bilirubin in rats fed with human milk. J Pediatr 1991; 118: 425-430.
6. Arias IM, Gartner LM, Seifter SA, Furman M. Prolonged neonatal unconjugated hyperbilirubinemia associated with breast-feeding and a steroid, pregnane-3 alpha, 20 beta-diol in maternal milk that inhibits glucuronide formation in vitro. J Clin Invest 1964; 43: 2037.
7. Erdem G, Öztürk R, Ciliv G, Özmert E, Tuncer M. Is beta-glucuronidase a contributory factor in early indirect hyperbilirubinemia. Acta Pediatr 1997; 86: 120.
8. Forsyth JJ, Donnet L, Ross PE. A study of the relationship between bile salts, bile salt stimulated lipase, and free fatty acids in breast milk: normal infants and those with breast milk jaundice. J Pediatr Gastroenterol Nutr 1990; 11: 205-210.
9. Freed LM, Moscioni D, Hamosh M, Gartner LM, Hamosh P. Breast milk jaundice revisited: no role for beta-glucuronidase or "unstimulated lipase". Pediatr Res 1987; 21: 267A.
10. Gaffney PT, Buttenshaw RL, Ward M, Diplock R. Breast milk beta glucuronidase and neonatal jaundice. Lancet 1: 1161-1162.
11. Gourley GR, Arend RA. Beta-glucuronidase and hyperbilirubinemia in breast fed and formula fed babies. Lancet 1986; 1: 644-646.
12. Hargreaves T. Effect of fatty acids on bilirubin conjugation. Arch Dis Child 1973; 48: 446-450.
13. İnce Z, Çoban A, Peker I, Can G. Breast milk β-glucuronidase and prolonged jaundice in the neonate. Acta Paediatr 1995; 84: 237-239.
14. Sirota L, Ferrera M, Lerer N, Dulitky F. Beta glucuronidase and hyperbilirubinemia in breast fed infants of diabetic mothers. Arch Dis Child 1992; 67: 120-121.
15. Wilson DC, Afrasiabi M, Reid mm. Breast milk beta glucuronidase and exaggerated jaundice in the early neonatal period. Biol Neonate 1992; 61: 232-234.
16. Scheider AP. Breast milk jaundice in the newborn. JAMA 1996; 255: 3270-3274.
17. Auerbach KG, Gartner LM. Breast-feeding and human milk: their association with jaundice in the neonate. Clin Perinatol 1987; 14: 89-107.