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Serum and Bile Bilirubin Pigments in the Differential Diagnosis of Crigler-Najjar Disease

ABSTRACT. ***Objective.*** **To differentiate between Crigler-Najjar (CN) disease types 1 and 2.**

Design. **The patterns of serum bilirubins, bile pigment composition, and phenobarbital response were studied.**

Patients. **Three infants, affected by high serum unconjugated bilirubin concentrations, previously classified as type 1 CN.**

Methods. **Serum and bile bilirubin pigment composition, both before and after phenobarbital (PB) treatment, were determined by alkaline methanolysis and high-pressure liquid chromatography. PB was given for at least 3 weeks by oral administration (5 mg/kg bw per day).**

Results. **No diconjugated bilirubin was found either before or after PB treatment in the serum of the three studied infants. In two patients traces of monoconjugated bilirubin were detected before PB therapy, and the ratio of conjugated/total bilirubin (percent) was increased by the PB response. In the third patient, traces of monoconjugated bilirubin appeared only after PB administration. However, the serum unconjugated bilirubin concentration decreased significantly only in the second patient, following the second cycle of PB treatment, leading to the diagnosis of type 2 CN. The analysis of the methyl ester derivatives of bile pigments was also performed on bile samples obtained in two patients by Entero-Test (R) both before and after PB treatment. An absolute increment in monoesterified bilirubin concentration was found after PB administration, although the percent concentration increased in one case and decreased in the other. No diesterified bilirubin was detected in the bile samples.**

Conclusions. **The present results show that in types 1 and 2 CN disease it is possible to detect traces of monconjugated but not diconjugated bilirubin both in serum and in bile. Whereas PB treatment is effective in slightly increasing the serum monoconjugated bilirubin concentration even in type 1 CN disease, the diagnosis of type 1 or 2 is based on finding a substantial decrease of serum unconjugated bilirubin following PB administration.** ***Pediatrics* 1994;94:553–556; *Crigler-Najjar disease, congenital hyperbilirubinemia, phenobarbital, pigment analysis, bile analysis.***

ABBREVIATIONS. CN, Crigler-Najjar; UCB, unconjugated bilirubin; PB, phenobarbital; AR, autosomal recessive; HPLC, high-performance liquid chromatography.

Crigler-Najjar (CN) disease is a rare disorder of bilirubin metabolism caused by a deficiency of hepatic UDP-glucuronyltransferase, and characterized by high serum levels of unconjugated bilirubin that appear in the first days after birth and continue through life.[1] The plasma unconjugated bilirubin (UCB) may increase to levels that exceed the binding capacity of plasma albumin, thereby causing kernicterus. Based on the responsiveness of the serum bilirubin concentration to treatment with phenobarbital (PB), CN disease can be distinguished in type 1, which does not respond to phenobarbital and is considered to be caused by a complete enzymatic defect, and type 2, which responds to PB and other drugs that induce enzyme synthesis and seems to be caused by a partial enzymatic deficiency. Type 1 is inherited as an autosomal recessive (AR) trait[2,3]; type 2 is also an AR condition,[2,3] although some authors advocate an autosomal dominant pattern of inheritance with variable penetrance.[4,5] At any rate, it is unquestionable that types 1 and 2 CN and Gilbert's syndrome are genetically related, because familial clustering of different forms of unconjugated hyperbilirubinemia are reported.[6,7] Furthermore, it is noteworthy that even type 2 CN, which is the less severe form, can cause neurological dysfunction in circumstances that cause increased production of bilirubin (eg, infections). Unfortunately, the differential diagnosis between types 1 and 2 is not always easy.[3,5] In fact, we have recently examined three infants affected by high serum unconjugated bilirubin concentrations, who had been previously classified as type 1 CN disease, because they apparently failed to respond to PB treatment. However, one subject was later found to respond to this agent and was thus reclassified as type 2. It should also be noted that accurate diagnosis is extremely important since orthotopic liver transplantation is an important therapeutic option in type 1 patients.

CASE REPORTS

Patient 1

F.A., 6 months old, came to our department on January 25, 1993 for treatment of jaundice caused by a presumptive Type 1 CN

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disease; the family history was negative. The patient was a 3650-g full term boy who was spontaneously delivered following an uneventful pregnancy. Phototherapy (12 hours a day) caused a reduction in the plasma UCB levels, but these tended to increase following suspension of treatment. PB (5 mg/kg bw/day) was initiated at 6 weeks of life but no improvement was noted. Upon admittance to our department the patient was in fair condition, and physical examination was negative except for severe cutaneous and mucosal jaundice. Extensive investigations failed to reveal any underlying diseases. The diagnosis of Type 1 disease was confirmed by alkaline methanolysis and high-performance liquid chromatography (HPLC) measurements[8] of the serum and biliary bilirubin levels (Table 1), which did not show any significant variation following a further trial with PB.

Patient 2

S.M., 22 days of age, came to our attention because of an intense jaundice, first noted during the second day of life in the community hospital where she was born. The patient was a full term girl who was spontaneously delivered following a normal pregnancy. No maternal use of medication or antepartum illnesses were reported. Physical examination was uneventful, except for the presence of jaundice. Suitable investigations excluded the presence of any other disease states; however, Gilbert's syndrome was diagnosed in the maternal grandmother. Phototherapy was initiated with the reduction of plasma UCB levels, but these tended to rise following suspension of treatment. PB therapy (5 mg/kg bw/day) was begun on the 15th day of life and continued for 3 weeks without any evident improvement; however, PB serum concentration was not monitored. During this period, the infant was transferred to the University Hospital. Subsequently, an experimental trial was undertaken with the intramuscular administration of Sn-protoporphyrin (2 μmol/kg of body weight), but this had to be interrupted due to the appearance of erythema during phototherapy, which was administered when the bilirubin serum concentration rose to about 20 mg/dL. Three months later PB was again attempted, and the positive response culminated in a definitive diagnosis of Type 2 disease (Fig 1). The serum and biliary bilirubin levels were monitored both before and after PB by means of the HPLC method[8] (Table 1). Phototherapy was suspended upon initiating a second trial with PB therapy, and plasma bilirubin levels have remained under control to date.

Patient 3

D.N.S. was a 3400-g full term girl spontaneously delivered after a normal pregnancy. No maternal use of medication or antepartum illnesses were reported. The father and a maternal great uncle were affected by Gilbert's syndrome. Jaundice was noted on the 2nd day of life, and the infant was treated with phototherapy; suspension of therapy caused a substantial increase of serum bilirubin concentration. Physical examination was unremarkable, except for the presence of jaundice. However, PB treatment (5

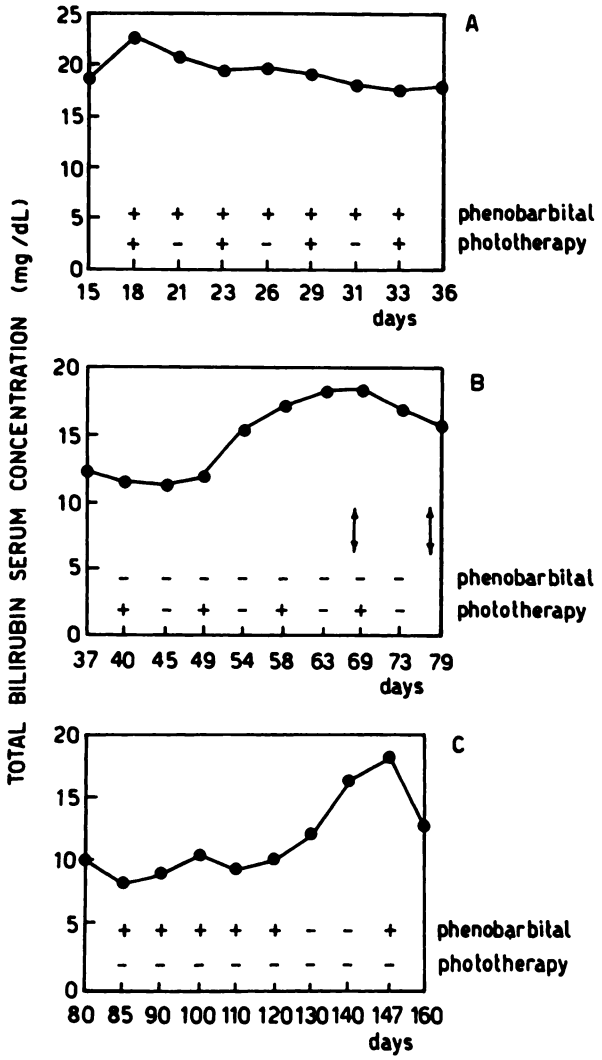

Fig 1. Serum concentration of total bilirubin (mg/dL) during different treatment regimens in patient 2. During the first period (A) the patient was treated with phenobarbital and phototherapy; during the second period she underwent phototherapy but phenobarbital was interrupted (B) and an experimental trial with Sn-protoporphyrin was undertaken (arrows indicate time of injection). During the third period (C), a new trial with PB was initiated which produced a positive response.

TABLE. Concentration of Unconjugated Bilirubin, Monoesterified Bilirubin, and Diesterified Bilirubin in Serum and Bile of Infants with Crigler-Najjar Disease

Patient	Type	Serum Pigment Concentration (μmol/L)		Biliary Pigment Concentration (μmol/L)	
		Before PB	After PB	Before PB	After PB
F.A.	1	UCB* = 83‡ MCB = 0 DCB = 0 CB/TB, % = 0	UCB = 137.7 MCB = 0.004 DCB = 0 CB/TB, % = 0.0005	UCB = 21.42 MCB = 5.15 DCB = 0 CB/TB, % = 19.39	UCB = 20.45 MCB = 7.99 DCB = 0 CB/TB, % = 28.1
S.M.	2	UCB = 282.48 MCB = 0.08 DCB = 0 CB/TB, % = 0.03	UCB = 154 MCB = 0.1 DCB = 0 CB/TB, % = 0.06	UCB = 2.5 MCB = 1.69 DCB = 0 CB/TB, % = 39.5	UCB = 22 MCB = 6.3 DCB = 0 CB/TB, % = 22
D.N.S.	1	UCB = 127‡ MCB = 0.175 DCB = 0 CB/TB, % = 0.14	UCB = 21.4‡ MCB = 0.17 DCB = 0 CB/TB, % = 0.79	ND	ND

* Abbreviations: UCB, unconjugated bilirubin; MCB, monoconjugated bilirubin; DCB, diconjugated bilirubin; PB, phenobarbital treatment; ND, not done.
‡ During phototherapy.

mg/kg bw/day) did not result in serum bilirubin decrement. The serum bilirubin concentration was assessed by alkaline methanolysis-HPLC[8] both before and after the PB trial (Table 1). At the age of 2.5 months, an experimental trial with Sn-protoporphyrin was undertaken, the results of which have been reported elsewhere.[7]

The patient eventually underwent successful liver transplant.

COLLECTION OF BILE AND BIOCHEMICAL ANALYSES

Bile samples were obtained by the pediatric Entero-Test (HDC Corporation, Mountain View, CA). Analysis of bile and serum samples, stored at −60°C, was performed within 1 month from collection. Alkaline methanolysis and liquid chromatographic analysis of bile and serum samples were performed according to Muraca and Blanckaert.[8]

The HPLC patterns of the methyl ester derivatives of pigments present in bile and serum of the three patients, as shown in Table 1, clearly demonstrate that unconjugated bilirubin was the predominant pigment both in the serum and in the bile. This is not surprising, because CN disease is characterized by a persistent unconjugated hyperbilirubinemia and, due to the effect of the phototherapy treatment, unconjugated bilirubin (ie, bilirubin isomers which then reconvert to the native pigment) is secreted in the bile. In patient 1, conjugated bilirubin was not found in the serum in the first bilirubin pigment profile (before PB), while about 20% of bilirubin present in the bile was monoconjugated. After PB therapy, a minimum amount of monoconjugated bilirubin appeared in the serum, while in the bile the presence of conjugated bilirubin increased to 28.1%. However, PB treatment did not maintain the serum bilirubin concentration permanently under 20 mg/dL. For this reason a diagnosis of CN disease type 1 was made.

In the serum bilirubin profile of the second patient, traces of conjugated bilirubin were present at the first examination. However, after PB therapy the percentage of bilirubin conjugates was still insignificant (0.06). The biliary pigment composition showed, at the first examination, a very modest concentration of unconjugated bilirubin and a relatively abundant percentage of conjugated pigment. After PB therapy, the levels of both unconjugated and monoconjugated bilirubin substantially increased, although the relative percentage of conjugated pigment was lower than before the PB treatment.

In the third patient, PB treatment slightly increased the percentage of monoconjuagted bilirubin in the serum from 0.14 to 0.79, but, also in this case, clinical correlation was unsatisfactory.

DISCUSSION

Type 1 CN disease is a very severe disorder entailing the risk of kernicterus. Therefore, it is extremely important to differentiate type 1 from type 2; in fact, liver transplantation is indicated in the former, while the latter is treated with PB.

It has been reported that bile pigment analysis can differentiate the two types:[3] in the absence of impaired biliary secretion, as a fraction of the esterified bilirubins formed in the liver normally refluxes from hepatocyte to plasma; therefore, determination of the esterified bilirubins in serum is of value in establishing the differential diagnosis of unconjugated hyperbilirubinemia.[8,9] In the present cases, small amounts of monoconjugated bilirubin were found before PB therapy in the serum of patients 2 and 3, and after PB treatment in the serum of patient 1. The percentage of serum conjugated bilirubin slightly increased during PB administration, but this observation was not in itself sufficient to permit discrimination between type 1 and 2 CN disease. In two patients a bile sample was obtained by Entero-Test both before and after PB treatment during a period in which phototherapy was suspended. An absolute increase in monoconjugated bilirubin concentration was found after PB administration, albeit the percent concentration (relative to total bilirubin) increased in case 1 and decreased in case 2. However, in case 2 the second course of PB treatment was effective in reducing the total serum bilirubin concentration, thus eliminating the need for phototherapy and allowing us to classify this form of congenital unconjugated hyperbilirubinemia as type 2 CN disease.

The reported results confirm a previous observation regarding type 1 disease, in which PB administration enhanced the levels of conjugated bilirubin in the serum but did not affect the concentration of unconjugated pigment assayed by diazoreactive methods.[5] We have demonstrated that, even in type 1 CN disease, in which the activity of UDP-glucuronyltransferase is below the threshold level of detection in the enzyme assay, PB administration is capable of inducing an increased serum and bile monoesterified bilirubin concentration. However, bile pigment analysis, performed with a very sensitive liquid chromatographic method,[8] does not differentiate between type 1 and 2. Only the clinical response to PB therapy differentiates the two types; however, it should be noted that during the first months of life a PB trial could be unsuccessful in the presence of type 2 CN disease.

It is noteworthy that diesters constitute about 80% of the total conjugates in normal human bile, although the percentage of diesters is lower in adult (50%) and neonatal (10%) human serum.[9,10] Diesters were undetectable in the serum and bile of our patients with CN disease, as previously reported by Muraca et al.[9] This observation suggests that, in transferase deficiency states, the formation of monoglucuronides predominates. This fact may be explained by the characteristic behavior of the microsomal bilirubin UDP-glucuronyltransferase system, wherein the major reaction product is bilirubin monoglucuronide in the presence of a high concentration of bilirubin substrate.[11] In fact, in serum of jaundiced neonates the concentration of diesterified bilirubin is increased significantly less than that of monoesters.[10]

From the reported data, it is not clear as to whether the increased amount of conjugated bilirubin found in the bile could explain the substantial decrement in the serum bilirubin level observed in case 2. It is possible to hypothesize that PB could decrease the serum bilirubin level not only by increasing UDPGT activity but also by inducing hepatic ligandin, as observed in jaundiced homozygous Gunn rats.[12] On

the other hand, the presence of monoconjugates in the bile of patient 1 and in the serum of patients 1 and 3, who were classified as CN disease type 1, may be explained by the presence of a residual UDP-glucuronyltransferase activity in the liver. This observation is not surprising, because, in CN type 1 individuals, the genetic defects are located at a different site in the UGT 1A gene, which suggests that many alterations in codons are likely to exist ranging from critical to moderately critical in terms of enzyme activity.[13]

In conclusion, whereas bile pigment analysis is useful in the diagnosis of CN disease, types 1 and 2 can only be differentiated on the basis of the response to PB treatment. It is noteworthy that, at least during the first months of life, the clinical response to PB can be insufficient even in type 2 CN disease, and a subsequent trial is at later date therefore advisable.

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An Infant in a Car Seat on a Washing Machine: Epidural Hematoma

Desperate parents resort to imaginative and sometimes dangerous improvisations to pacify a crying infant. One measure that has actually received physician endorsement in popular parenting publications is to set the inconsolable baby, often suffering from colic, in a car seat on an engaged clothes dryer or washing machine.[1–3] The warmth, rhythmic noise, and vibration together have the desired soporific effect. As the following case report illustrates, placement of car seats on vibrating elevated surfaces can have life-threatening side effects as well.

CASE REPORT

A 7-month-old male was brought by ambulance to the emergency room after suffering an apparent seizure. Approximately 5 hours prior to admission, the infant was placed on an engaged washing machine in his car seat while his parent was starting the car. The infant was found moments later on the floor, crying. He had no apparent injury and otherwise behaved normally. Within the next 2 hours, the infant became more irritable, vomited once, and appeared listless. He then had a generalized seizure and an ambulance was called.

Physical examination upon admission demonstrated a well developed 7-month-old whose mental status waxed and waned from crying to lethargy. Intermittent bradycardia was noted. A right parietal scalp hematoma was present. There were no neurologic abnormalities.

A head computerized tomographic (CT) scan showed a right frontal epidural hematoma with a midline shift (Figure). The

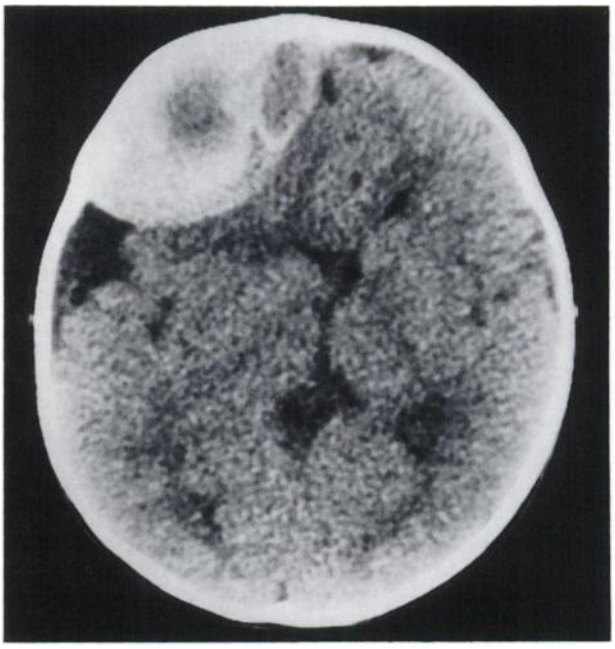

Figure. A head CT scan showing a right frontal epidural hematoma with a midline shift.

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