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DELTA BILIRUBIN IN VARIOUS FORMS OF JAUNDICE

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RESUME

La bilirubine delta (DELB), également connue comme biliprotéine, est une fraction de bilirubine moins utilisé dans la pratique médicale en raison de l'insuffisance des méthodes appliquées dans certains laboratoires. L'analyseur Vitros 950 pour la biochimie sèche, donc l'Hôpital Clinique d'Urgence pour les Enfants «St. Jean» du Galatzi est doté, est capable de mesurer les fractions indépendantes de bilirubine et d'estimer la valeur de la bilirubine delta, sa présence dans le sérum se corrélant, selon la littérature, avec le diagnostic de l'ictère obstructif. Pendant les années 2005-2010, DELB a été remarqué chez 57 enfants traités dans notre hôpital, 30 filles et 27 garçons âgés de 1-17 ans. Quand la bilirubine indirecte est en excès (plus de 80% bilirubine totale), il a été constaté que la bilirubine directe est constituée, presque entièrement, de DELB (16 cas). Pour 16 malades, avec une augmentation proportionnelle de la bilirubine non conjuguée et conjuguée, DELB représente aussi un pourcentage significatif de bilirubine directe. Quand le taux de bilirubine directe monte plus de 50% de la bilirubine totale, correspondant aux phénomènes d'obstruction biliaire, la concentration de bilirubine delta est environ la moitié de DBIL, avec un rapport DELB / bilirubine totale inférieure à 0,3. DELB est une fraction de la bilirubine avec demi-vie prolongée dans le sang qui est associée à des phénomènes obstructifs persistants. Comme indicateur de dysfonctionnement hépato-biliaire, sa détermination est recommandée dans le diagnostic et le suivi de l'ictère cholestatique, y compris les enfants.

MOTS CLES*: fraction de bilirubine, bilirubine delta, ictère obstructif*

1.Introduction

Jaundice is a pathological state caused by excessive bilirubin. The type of jaundice is established through routine measurements of indirect bilirubin, direct bilirubin (DBIL) and total bilirubin (TBIL), as well as its variety, neonatal bilirubin (NBIL). Some modern analyzers can also measure conjugated bilirubin, thereby revealing a lesser known fraction, δ bilirubin or biliprotein (DELB). Bilirubin is an intermediary pigment from heme catabolism [1]. Protoporfirine IX from heme is converted by heme oxygenase mostly into the biliverdin IXα isomer [5,8] As a result, most of the bilirubin generated from the iron catabolism is in the form of the IXα isomer, also known as α/indirect bilirubin. Bilirubins mono- and diglucuronide, generated in the liver (named, respectively, β and γ), represent conjugated bilirubin (Bc). Consequently, indirect bilirubin is also known as unconjugated bilirubin (Bu). In some hepatobiliary dysfunctions, Bc binds nonenzymatically to albumins using glucuronide and forming a complex called DELB. Bc and DELB forms

direct bilirubin, a product which exhibits prolonged halftime in blood [4,9]. Proportional increases in Bu and DBIL fractions signal intrahepatic afflictions of unknown origins. "Retention" hyperbilirubinemia [10], which occurs at Bu levels above 80% TBIL, indicates a prehepatic cause for jaundice, while "regurgitation" hyperbilirubinemia (ibid.), when DBIL > 50% TBIL, are commonly associated with posthepatic jaundice [7]. Obstruction phenomena were found to be accompanied by increases in serum DELB, which cannot be excreted through urine because of their protein binding. This aspect is considered responsible for persistent hyperbilirubinemia and also lack of bilirubinuria in cholestatic cases. In newborns, DELB levels over 50% of TBIL were associated with intra- and extra-hepatic cholestasis, biliary atresia and hepatitis. In children, DELB was found accompanying approximately 10% of cases of hemolytic anemia, sepsis, shock and non-hepatic jaundice [3].

The aim of this study was to enforce the clinical importance of evaluating DELB, which may aid in the dynamic quantitative characterization of biliary obstructive phenomena even in early ages.

2. Materials and methods

In "St.John" Clinical Emergency Children's Hospital of Galati, bilirubin fractions are measured and computed using *MicroSlides* methode in dry biochemistry analyzer *Vitros 950*, as follows:

-Bu (previously dissociated from the albumin using caffeine and sodium benzoate) and Bc are bound to a cationic mordant which separates the reflectance maxima of conjugated and unconjugated bilirubin. Absorption is measured at two wavelengths: 400 and 460 nm. This special method was developed by Eastman Kodak in early 1980s [15,16].

-TBIL is determined colorimetrically based on a modification of the classic diazo reaction [12], by measuring the absorbance of azobilirubin cromophores, formed in the reaction between a diazonium salt and Bu (enzimatically dissociated from albumin), Bc and DELB, at two different wavelengths (540 and 460 nm);

-NBIL is calculated as the sum of Bu and Bc [13];

-DBIL is calculated as the difference between TBIL and Bu;

-DELB is calculated as: TBIL – (Bu + Bc).

Between 2005 and 2010, DELB was found in 57 children (30 girls and 27 boys) treated in "St. John" Clinical Emergency Children's Hospital of Galati. The average age in the patient group was 9.28±6.09 years, respectively 9.6±5.8 years for girls and 8.93±6.49 years for boys.

3. Results and discussions

TBIL was averaged over the investigated group at 3.03±4.44 mg/dL (with respective values of 0.85±1.71 mg/dL in girls and 3.71±6.11 in boys), the DBIL average was 1.23±1.58 mg/dL (1.18±1.29 mg/dL in girls and 1.26±1.91 mg/dL in boys) while the Bu averaged at 1.59± 3.51 mg/dL (1.09±0.80 mg/dL in girls and 2.22±5.01 mg/dL in boys). Standard deviations indicated a more homogenous distribution of the analyzed values in girls, as compared to boys. The larger distribution interval for male patients is largely due to Bu values.

In 16 subjects (7 girls and 9 boys), indirect bilirubin represents over 80% of total bilirubin and is associated with anemia in just two patients (one 4-year old female and a newborn male). Excessive Bu concentration (above 80%) may be correlated with either a liver incapacity to conjugate it, due to a decrease/absence of glucuronil transferase activity, or an accentuated hemolysis, when the liver's detoxification ability is overwhelmed by the concentration of toxin (Bu). Increased Bu may also occur when administering glucuronid competitive medication (e.g. paracetamol). A differential diagnosis for "retention" hyperbilirubinemia is established based on hemoglobin levels. At normal

hemoglobin levels (above 12 g/dL) a liver enzyme deficit is usually to blame for the condition. The deficit may be total or partial in genetic disease such as Crigler-Najjar syndrome or Gilbert syndrome, which manifests with uridindifosfat-glucuronil transferase gene mutations that diminish enzyme activity [12]. Indirect hyperbilirubinemia may also be associated, if hemoglobin is normal, with organic dysfunctions in liver uptake or septicemia. When indirect bilirubin rises above 80% of total bilirubin, while hemoglobin stays below 12 g/dL and reticulocyte levels rise, the diagnosis should be oriented towards diseases that imply hemolysis: hemolytic anemia, thalassemy, pernicious anemia or inefficient erythropoiesis [5,6].

Direct bilirubin was above 50% of total bilirubin in 25 patients – 13 girls with an average age of 6.85±5.64 years and 12 boys averagely aged 8.25±6.80. Two of these subjects (one male and one female) exhibited anemia and 12 (6 male and 6 female) had high hepatic enzyme activity, as associated with viral hepatitis. Since serum should not contain conjugated bilirubin (therefore neither DELB), direct bilirubin is an extremely sensitive marker for hepatic dysfunction and is due to biliary flow anomalies, usually caused by obstructions (calculi or tumors) of the biliary tract or genetics (Rotor or Dubin-Johnson syndrome) [11].

Table 1. Direct bilirubin and biliprotein averages in study subgroups

Bilirubin fraction ratios	DBIL (mg/dL)		DELB (mg/dL)	
	female	male	female	male
Bu > 80% TBIL	0.26±0.14	0.23±0.10	0.23±0.15	0.30±0.38
DBIL > 50% TBIL	2.08±1.24	2.04±2.50	0.67±0.34	0.88±0.82
DBIL<50% TBIL Bu < 80% TBIL	0.73±0.94	0.74±0.87	0.40±0.30	0.45±0.21

In the remaining 16 patients (6 male and 10 female) Bu and DBIL values were outside previously specified intervals (average ages were: 11.70±5.72 years in girls and 7.67±5.28 years in boys). No pathological hemoglobin or liver enzyme level anomalies were detected in this group. The calculated values for DBIL and DELB are listed in Table 1.

The calculated averages for DBIL and DELB fractions are relatively similar for both sexes. When indirect bilirubin was specific to prehepatic jaundice, direct bilirubin was almost entirely made up of delta bilirubin (DELB/DBIL=0.87). In the case of intrahepatic jaundice, delta bilirubin also made up a large part of direct bilirubin (DELB/DBIL=0.79). However, when excessive bilirubin was associated with posthepatic obstructive phenomena, DELB/DBIL ratios dropped significantly (to an average of 0.55).

Considering the persistance of delta bilirubin in blood and the correlation between DELB levels and biliary drainage reestablishment, determining the DELB/TBIL ratio becomes useful for characterizing obstructive phenomena [3,6]. Values for DELB/TBIL ratios calculated for this study are depicted in Figure 1 and associate elevated DELB with post-hepatic causes for hyperbilirubinemia and biliary obstructive phenomena. DELB/TBIL ratios below 0.3 were found in 25 members of the study lot, while the rest exhibited higher levels of up to 0.84.

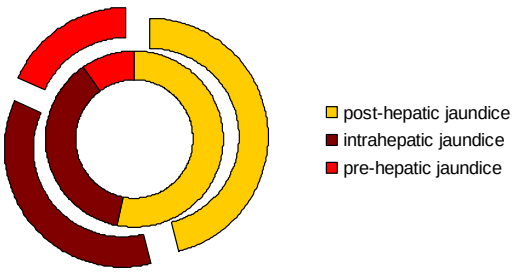

Figure 1. *Average DELB/TBIL ratios in study lot*

5. Conclusions

By measuring and correlating bilirubin fractions, cases of jaundice in the study lot were attributed to pre-hepatic (28.07%), post-hepatic (43.85%) and unknown

intra-hepatic causes (28.08%). Pre-hepatic jaundice was associated with hemolytic anemia in only two cases, while 14 hyperbilirubinemias exhibited normal hemoglobin levels. Biliary obstructive phenomena were associated with two cases of anemia and 12 cases of hepatitis.

Various concentrations of biliprotein were measured in all cases of jaundice diagnosed according to recommendations in *„Interpretation of Diagnostic Tests"* by Jacques Wallach.

Biliprotein was identified in serum from patients of all ages, including newborns.

Large concentrations of delta bilirubin indicate long term cholestasis. Although DELB/TBIL ratio evolutions are relevant for obstructive jaundice, DELB dynamics could not be studied because of reduced awareness about biliprotein and its role. Therefore, it is the authors' hope that this study will spark further interest and constitute a basis for future studies.

DELB is a good indicator of liver and bile function and is a recommended test in the diagnosis and monitoring of cholestatic jaundice, a condition which also occurs in children.

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