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Investigation of Total and Conjugated Bilirubin Determination during the Neonatal Period

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Summary: During the neonatal period, total and conjugated bilirubin determinations are necessary to identify the origin of jaundice, to predict its evolution and to treat it. We discuss the results obtained in 108 neonates (less than 15 days old), undergoing phototherapy or not, using a colorimetric diazo reaction and dual wavelength reflectance with a Kodak Ektachem analyzer. Concerning total bilirubin determination, the methods correlate well ($r > 0.96$). Discrepancies are observed for conjugated or “direct” bilirubin, and high performance liquid chromatography was carried out in order to explain them. The chromatograms show 4 neonate samples with only classic mono- but no di-glucurono-conjugate fractions, whereas all the neonates present two unusual fractions (I and II) not seen in adults. A correlation was found between the amount of fraction II and the conjugated bilirubin determined by diazo reaction and between fraction I and the conjugated bilirubin obtained in the Kodak Ektachem assay. A better correlation between fraction I and conjugated bilirubin on Kodak was observed ($r = 0.79$, vs $r = 0.66$) when the newborns were submitted to phototherapy. Moreover, fraction II and conjugated bilirubin measured by the diazo reaction on Hitachi 717 rose significantly.

In conclusion, total bilirubin is accurately determined during the neonatal period; for conjugated or “direct” bilirubin determination, our study points out significant differences. Further investigation will determine the nature of the fractions observed by liquid chromatography in neonatal sera, and the components actually determined by the automatized methods usually employed.

Introduction

Neonatal hyperbilirubinaemia is currently observed during the neonatal period (25–50% of all neonates), mostly due to low activity of the hepatic glucuronosyltransferase (EC 2.4.1.17) (1). Total and conjugated (or “direct”) bilirubin determinations are necessary to identify the origin of the jaundice, to predict its evolution and to treat it. Visible light phototherapy is one of the effective treatments frequently given to these children, but it produces photochemical prod-

ucts (2) that may interfere with the biochemical methods commonly used for bilirubin determination (3).

The aim of this study is to compare the data of total and conjugated bilirubin measured in neonates, undergoing phototherapy or not, using two commonly employed methods: colorimetric diazo reaction on a Hitachi 717, dual wavelength reflectance on a Kodak Ektachem 700. High performance liquid chromatography was then used for further investigation.