Unconjugated bilirubin in human bile: the nucleating factor in cholesterol cholelithiasis?

M K Dutt, G M Murphy, R P H Thompson

Aims: To investigate the concentrations of bilirubin, bilirubin conjugates, phospholipid, and cholesterol in the gall bladder bile obtained at surgery from patients with and without cholesterol gallstones.

Methods: Gall bladder bile was collected during surgery, by puncture, from 20 patients with gallstones undergoing routine cholecystectomy and from eight patients with normal liver blood tests. Concentrations of bilirubin, bilirubin conjugates, phospholipid, and cholesterol were measured using standard procedures.

Results: The proportion of total bilirubin that was unconjugated was significantly higher in the bile from patients with stones than in bile from control patients, whether or not the bile from either group was saturated with cholesterol or not. Indeed, the mean concentration of cholesterol was significantly higher in control bile samples.

Conclusion: The presence of stones was more closely related to the proportion of unconjugated bilirubin than to the degree of saturation of bile with cholesterol. Bilirubin and its metabolites probably play an important part in the formation of cholesterol gallstones.
to the CSI (table 1). Indeed, the mean biliary cholesterol, phospholipid, and total lipid concentrations in the stone group were significantly lower than those in the control group (p < 0.02; table 1).

The major bands of bilirubin detected in all samples were bilirubin IX C-8 monomethylester (Rf 0.5), bilirubin IX C-12 monomethylester (Rf 0.45), and bilirubin dimethylester (Rf 0.25). Minor bands were bilirubin III and XIII monomethyl-esters (Rf 0.54 and Rf 0.01, respectively).

The biliary total and unconjugated bilirubin concentrations were not significantly different between the two groups (fig 1). However, the unconjugated fraction expressed as a proportion of the total bilirubin present was significantly higher in the cholesterol gallstone group: mean, 3.41%; median, 3.04%; SD, 1.4%; n = 20; controls: mean, 1.74%; median 1.51%; SD, 0.8%; n = 8; p < 0.002). The mean unconjugated bilirubin fraction was also higher in the stone group, although the difference was not significant (cholesterol gallstone group: mean, 26.56%; median 22.5%; SD, 9.3%; n = 18; controls: mean, 17.67%; median, 18.0%; SD, 6.9%; n = 6; p = 0.062).

Therefore the presence of stones correlated more with the proportion of unconjugated bilirubin than with the CSI. Indeed, in the 15 patients with cholesterol gallstones and bile saturated with cholesterol, the mean percentage of bilirubin present in the unconjugated fraction (mean, 3.33%; median, 2.84%; SD, 1.5%; n = 15) was higher than that in the four control subjects also with cholesterol saturated bile but without gallstones (mean, 1.63%; median, 1.51%; SD, 0.8%; n = 4; p < 0.02), and also higher, but not significantly different, from that in the other four control subjects whose bile was not saturated with cholesterol (mean, 1.85%; median, 1.72%; SD, 0.8%; n = 4; 0.05 > p < 0.10).

In the five patients with cholesterol gallstones but bile unsaturated with cholesterol, the percentage of bilirubin present in the unconjugated fraction (mean, 3.66%; median, 3.25%; SD, 1.4%; n = 5) was significantly higher than that seen in the eight subjects without gallstones, whether their bile was saturated with cholesterol (mean, 1.63%; median, 1.51%; SD, 0.8%; n = 4; p < 0.04) or not (mean, 1.85%; median, 1.72%; SD, 0.8%; n = 4; p < 0.04; fig 2).

DISCUSSION

Our results confirm that the CSI and lipids in the gall bladder bile of patients with gallstones are not consistently higher than in controls, although the bile of patients with cholesterol gallstones frequently contains a greatly increased proportion of unconjugated (and also probably of monoconjugated) bilirubin. Such observations are in accordance with the hypothesis that deconjugation of bilirubin glucuronides in bile may be an early event in the formation of cholesterol gallstones with a pigmented nidus, rather than a primary abnormality of lipid metabolism being the cause.

The major bilirubin metabolite in human bile is its diglucuronide, which, together with the monoglucuronide, accounts for more than 98% of total biliary bilirubin. Deconjugation may be catalysed by glucuronidases of bacterial or mucosal origin, and bilirubin monoglucuronide may also undergo non-enzymatic hydrolysis to form unconjugated bilirubin. Even in normal bile, the low concentration of unconjugated bilirubin still exceeds its aqueous solubility 100–1000-fold, so that bile has frequently been described being supersaturated with calcium salts of bilirubin.

Increased proportions of unconjugated bilirubin in gallbladder bile may be a consequence of impaired gallbladder motility. Prolonged storage of bile in the gallbladder, in addition to increasing the length of exposure of conjugated bilirubin to glucuronidases, may also decrease the biliary pH, although this has not been measured in humans. Endogenous β-glucuronidase activity can be detected at pH 7.5, but its optimum pH is 4.5–5.0, so that a lower pH would favour the formation of unconjugated bilirubin. Even in sterile gallstones, bacterial DNA can usually be detected, suggesting
Take home messages

- The presence of stones was more closely related to the proportion of unconjugated bilirubin than to the degree of saturation of bile with cholesterol.
- Bilirubin and its metabolites probably play an important part in the formation of cholesterol gallstones.

that bacterial glucuronidases have been active.22 Unconjugated bilirubin has been identified as a component of biliary sludge, which is believed to precede gallstones,23,24 particularly pigmented stones.25,26

“The proportions of conjugated to unconjugated and/or monoconjugated bilirubin may be important in the nucleation of cholesterol gallstones.”

The concept that unconjugated bilirubin plays at least as an important role in the formation of cholesterol gallstones as does cholesterol saturation is not new,23,26 but has received little attention. Our finding of an increased proportion of bilirubin in its unconjugated moiety in the bile of patients with cholesterol gallstones rather than an increase in the total bilirubin concentration has previously been observed mainly in bile containing pigmented stones,25,26 but we found that unconjugated bilirubin is a feature of both cholesterol saturated and unsaturated bile from patients with cholesterol gallstones, although this conclusion is based on small numbers. Hence, there was a better correlation between the proportion of unconjugated bilirubin and the presence of stones than the classic cholesterol saturation index, raising the possibility that the proportions of conjugated to unconjugated and/or monoconjugated bilirubin may be important in the nucleation of cholesterol, or that it is simply a marker of glucuronidase activity related to poor gall bladder motility. Many studies have emphasised the roles of impaired motility of the gall bladder27-29 and of the intestine30 in the pathogenesis of gallstone disease, and impaired gall bladder motility would favour the formation of unconjugated bilirubin. There is no agreement on the relative importance of these factors,31 but our results add to the hypothesis that unconjugated bilirubin plays an important role in cholesterol precipitation (M K Dutt. MD Thesis, University of London, 1983).

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