Copper in urine and hair in Indian childhood cirrhosis

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SUMMARY In advanced Indian childhood cirrhosis (ICC) urine copper concentration was higher (range 416–103 448 mg/g creatinine) than in other hepatic diseases (range 67–10 303 mg/g creatinine). In early ICC urine copper concentration was more modestly raised (1188–9470 mg/g creatinine), but rose to high values (2222–42 819 mg/g creatinine) after a single dose of penicillamine 20 mg/kg. A post-penicillamine urinary copper:creatinine ratio >10 000 mg/g supports a diagnosis of ICC. The concentration of copper in the hair, while increased in advanced ICC, is of no diagnostic value in early cases.

The clinical diagnosis of Indian childhood cirrhosis (ICC) is easy in the late presenting case but is unreliable at an earlier stage. Liver biopsy is therefore necessary.¹ We sought a robust non-invasive diagnostic method and we have therefore evaluated copper concentrations in urine and hair. To obviate the need for 24 hour urine collections, which would not be feasible if the method was to become of value in the field, the copper:creatinine ratio was measured in random samples of urine. The cupriuretic effect of a single dose of penicillamine is of diagnostic value in Wilson’s disease,² and was studied in ICC and other hepatic disorders. Hair is painless to collect, easy to store and transport, and provides a historical measure of intracellular copper. Despite well described limitations of hair trace metal analysis,³ previous workers have suggested that copper concentration in hair is raised in ICC.⁴ ⁵

Methods

Urine was collected from 57 children with ICC and
from 21 children with other hepatic disorders (table) in Pune.\textsuperscript{1} The children studied were part of a prospective review of liver disease, diagnosis being confirmed by a liver biopsy specimen in each case. Urine was stored at 4°C in plastic containers and transported in batches to Leicester.

Eight children (aged 4 to 48 months) with early ICC—that is, without jaundice or ascites—and 15 children (aged 3 to 48 months) with other hepatic disorders (table) had urine collected before and 24 hours after an oral dose of penicillamine 20 mg/kg body weight.

Urine, with added ammonium nitrate to give a final concentration of 5\% to cause sodium chloride volatilisation, was assayed directly for copper by electrothermal atomic absorption spectrophotometry (Pye Unicam SP9).

Containers were shown to be copper free by assaying acid washings. The extent to which containers took up copper from samples (n=10) was found to be insignificant at 0-5 (SD 0-37)\%. Repeated assay of an aliquotted urine over a three year period showed good reproducibility (7-32, (0-95) \(\mu\)mol/l urine). Urinary creatinine concentration was determined colorimetrically.\textsuperscript{6}

Hepatic copper concentrations in percutaneous biopsy specimens were assayed as previously described.\textsuperscript{1} Hair was taken from 40 cases of ICC (age 2–48 months); 18 Pune patients with non-hepatic disorders (age 10–48 months); and 15 Leicester inpatients (age 1–48 months). Snippings were taken from the crown and the entire length assayed. To remove surface contamination samples were washed with 4 ml acetone followed by 4 ml deionised water and then the washings repeated; each wash took 10 minutes. Samples were dried, weighed, and then digested in 2 ml of 50\% nitric acid at 80°C for three hours. After evaporation to dryness samples were redissolved in 2 ml of 0-1\% nitric acid. Copper was assayed as above.

A sample of hair repeatedly assayed gave results of 22-6 (9-1) \(\mu\)g copper/g hair.

Clinical, laboratory, and histological variables were recorded as previously described.\textsuperscript{1} Values for copper:creatinine ratio showed a skewed distribution and were log transformed for analysis. Groups were compared using Student’s unpaired \(t\) test.

### Results

The urinary copper:creatinine ratio in 57 children with ICC was significantly greater than that found in 21 children with other hepatic disorders despite the wide scatter results (p<0-001). Among children with ICC, more advanced disease was associated with higher copper:creatinine values. Ascites and jaundice are late features of ICC, and cases with neither jaundice nor ascites (n=15) had a copper:creatinine ratio with a significantly lower range (416–39 600,

### Table

Mean (SD) hepatic copper concentration and urinary copper:creatinine ratio in 57 children (11 girls, 46 boys) with ICC and in 21 children with other hepatic disorders. Urinary copper:creatinine ratio was also assayed in eight children with early ICC and in 15 children with other hepatic disorders before and 24 hours after a single dose of penicillamine 20 mg/kg

<table>
<thead>
<tr>
<th>No of children</th>
<th>Hepatic copper ((\mu)g/g dry weight)</th>
<th>Urinary copper:creatinine ratio (mg copper/g creatinine)</th>
<th>Median</th>
<th>Range</th>
<th>(\text{Log}_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC</td>
<td>57</td>
<td>1667 (694)</td>
<td>6781</td>
<td>416–10 448</td>
<td>3-92 (0-52)†</td>
</tr>
<tr>
<td>Other hepatic disorders</td>
<td>21</td>
<td>383 (373)</td>
<td>452</td>
<td>67–10 303</td>
<td>2-72 (0-58)†</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>6</td>
<td>498 (309)</td>
<td>282</td>
<td>75–10 303</td>
<td>2-70 (0-73)</td>
</tr>
<tr>
<td>Chronic persistent hepatitis</td>
<td>5</td>
<td>162 (42-8)</td>
<td>245</td>
<td>67–3025</td>
<td>2-65 (0-70)</td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td>1</td>
<td>853</td>
<td></td>
<td>3-0</td>
<td></td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>1</td>
<td>1250</td>
<td></td>
<td>3-0</td>
<td></td>
</tr>
<tr>
<td>Others*</td>
<td>8</td>
<td>219 (313)</td>
<td>681</td>
<td>77–2000</td>
<td>2-73 (0-54)</td>
</tr>
</tbody>
</table>

**Penicillamine trial**

|ICC Before and 24 Hours after| 8                                    | 1342 (1037)                                           | 4311   | 1188–9470 | 3-65 (0-30)$£ |
|Other hepatic disorders Before and 24 Hours after| 15                                   | 453 (378)                                             | 288    | 67–10 303  | 2-74 (0-60)$£ |

*Other disorders: extrahepatic biliary atresia (n=2), infective hepatitis (n=2), glycogen storage disease (n=1), tuberculosis (n=1), syphilitic hepatitis (n=1), and minor changes (n=1).

†p<0-001; ‡p<0-006; $p<0-001.
log₁₀ 3.7 (0.5) than those with both jaundice and ascites (1643–91707, log₁₀ 4.1 (0.5)) (p<0.011). No correlation existed, however, between the copper:creatinine ratio and serum total or conjugated bilirubin, serum transaminases, or hepatic copper.

In early ICC urine copper concentrations were more modestly raised and showed overlap with those found in other liver disorders even though mean values were significantly different (table). After a single oral dose of penicillamine 20 mg/kg a large rise in urinary copper occurred in children with ICC to values exceeding 10 000 mg/g in seven of eight children. A considerably smaller rise occurred in other hepatic disorders, the post-penicillamine value exceeding 10 000 mg/g in only two children. One of these had Wilson’s disease and one had chronic active hepatitis (hepatic copper concentrations being 853 and 508 µg copper/g dry weight respectively, normal <100 µg/g).

Concentrations of copper in the hair of children with ICC (71.9 (55.9), range 13–220) was higher than in the controls from either the United Kingdom (25.8 (14.6), range 11–69) or India (58.7 (39.7), range 16–170) but the wide scatter of results made these differences non-significant. Children with ICC that was clinically judged to be early had a significantly lower hair copper concentration (19.0 (4.3)) than later (92.1 (86.0)) or terminal cases (93.0 (53.0)). There was no correlation between hepatic and hair copper.

Discussion

The present data show that the hepatic copper storage characteristic of ICC is accompanied by greatly raised urine copper:creatinine ratios. In advanced cases values of 10 000–93 000 mg/g were found. Absolute concentrations of copper in the urine were 20.5–856.3 µmol/l, concentrations readily detectable by standard chemical assays. Random urine copper is therefore of some diagnostic value, particularly when coagulation defects make liver biopsy hazardous.

It is, however, in the early case that non-invasive diagnostic methods are more urgently required. The present study shows that in ICC that has not progressed to the development of ascites or jaundice urinary copper:creatinine ratios are modestly raised and do not provide a clear distinction from other hepatic disorders. After a single dose of penicillamine 20 mg/kg, however, the ratio sharply increased. Values exceeded 10 000 mg copper/g creatinine in seven out of eight cases. Other disorders associated with raised hepatic copper show a modest post-penicillamine cupriuresis. The other important cause of raised urine copper in infancy, extrahepatic biliary atresia, is clinically quite distinct from ICC, however, while Wilson’s disease is rarely symptomatic before 5 years of age, and may be differentiated from ICC by serum caeruloplasmin assay.

Hair copper concentrations are raised in advanced ICC confirming previous reports, but the diagnostic value of this is limited because of the variation in normal values. Exogenous copper contamination contributes to this variation despite meticulous sample preparation. The difference between the controls in the United Kingdom and India may reflect different use of oily scalp preparations or may genuinely reflect copper status. Another disadvantage is that hair copper concentration is normal in the early case in which diagnosis is most difficult.

We conclude that random urine and hair copper concentrations are raised in advanced ICC. While hair copper is of no diagnostic value in early ICC a post-penicillamine urine copper:creatinine ratio greater than 10 000 mg/g is highly suggestive of the diagnosis.

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References


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