Present interpretation of the role of copper in Indian childhood cirrhosis1–3

Anand Pandit and Sheila Bhave

ABSTRACT A common killer disease of the past, Indian childhood cirrhosis (ICC), which became preventable and treatable in the early 1990s, is now rare. ICC must be clearly distinguished in Indian children from other chronic liver disorders including Wilson disease. Grossly increased hepatic, urinary, and serum copper concentrations are characteristic of ICC. These increased concentrations are easily demonstrated histologically with orcinorhodanine staining. Environmental ingestion of copper appears to be the most plausible explanation for ICC, as shown by feeding histories, the prevention of ICC in siblings and in the Pune district by a change in feeding vessels, and the dramatic reduction in incidence of ICC throughout India. The nature and role of a second factor in the causation of ICC remains unclear, although an inherited defect in copper metabolism is strongly suspected. ICC, however, does not appear to be a straightforward early onset of Wilson disease because ceruloplasmin is consistently normal and clinical and histologic recovery is maintained in the long term despite withdrawal of d-penicillamine therapy. Descriptions of an ICC-like illness in the West suggest that different mechanisms (environmental, genetic, or both) can lead to the same end-stage liver disease: copper-associated childhood cirrhosis. ICC probably represents a specific form of copper-associated childhood cirrhosis that requires high environmental copper ingestion for its full expression. Am J Clin Nutr 1996;63:830S–5S.

KEY WORDS Indian childhood cirrhosis, copper-associated childhood cirrhosis, copper toxicosis

INTRODUCTION

Indian childhood cirrhosis (ICC) has fascinated the scientific community for at least 100 y (1). The peculiar features, the enigmatic etiology, and the uniformly fatal outcome have frustrated many (2–4). In 1978 a finding almost by chance revealed a striking association of exceedingly high hepatic copper concentrations with ICC (5, 6). This exciting finding (subsequently confirmed by many) led to a viable hypothesis of copper-contaminated milk feeds as a cause of ICC and a suggested means of treating and even preventing the disease (7–11). The subsequent dramatic reduction in the incidence of ICC throughout India during the past two decades has been particularly satisfying (12).

Whereas ICC is disappearing in India, scattered reports of ICC-like cirrhosis are increasingly appearing in Western countries (13–16). It is unclear whether this disease (or diseases) is similar to or different from ICC as seen in India. It seems important, therefore, to have a clear understanding of ICC and to critically reappraise the role of copper in the pathogenesis of ICC. More than 1000 children with chronic liver disease (including 300 with ICC) have been assessed at our center at the KEM Hospital in Pune during the past 15 y (Table 1). The following review is based largely on our own studies in collaboration with MS Tanner (Leicester University, United Kingdom, now at Sheffield, United Kingdom), although we also refer to contemporary literature on ICC.

WHAT IS INDIAN CHILDHOOD CIRRHOSIS?

The characteristic clinical and epidemiologic features of ICC are as follows (17–20). 1) The specific age range is from 6 mo to 5 y with a mean of 18 mo. 2) The disease is predominantly in males. 3) There are high rates of parental consanguinity in families affected with this disease, and up to 22% of siblings are affected. 4) The disease is restricted to the Indian subcontinent, and the origin of cases is rural rather than urban. 5) Distribution by religion and caste reflects the local rural population, but Muslims and Christians are mostly spared. 6) The onset is mainly insidious (86%) with nonspecific complaints such as abdominal distention, irregular fever, excessive crying, and altered appetite. In a few children, the disease begins with jaundice, but this is usually a late feature. The feel of the liver is characteristically firm to hard with a sharp, “leafy” edge. Untreated, the progress is relentless and within a few months the affected child is desperately ill with hepatosplenomegaly, ascites, edema, and jaundice. An unusual late feature is an enlarged, palpable gall bladder, believed to be due to cholangitis and cholecystitis. Death is usually due to bleeding, secondary infection, or hepatic coma. 7) Standard liver function tests are usually abnormal but not diagnostic. Clinical or biochemical differentiation of early ICC from other childhood liver disorders such as unresolved viral hepatitis, chronic persistent or active hepatitis, veno-occlusive disease, and cryptogenic cirrhosis is difficult and hence histopathology remains the cornerstone of definitive diagnosis.

1 From the Department of Pediatrics, KEM Hospital, Pune, India.
2 Supported by the Wellcome Trust, United Kingdom.
3 Address reprint requests to S Bhave, Department of Pediatrics, KEM Hospital, Pune 411 011, India.

TABLE 1
Pattern of chronic liver disease in 849 children biopsied at KEM Hospital in Pune1

<table>
<thead>
<tr>
<th>Chronic liver disease</th>
<th>Average annual incidence</th>
<th>no. cases/10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian childhood cirrhosis</td>
<td>47</td>
<td>23</td>
</tr>
<tr>
<td>Infantile cholangiopathy</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Wilson disease</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Storage diseases</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Hepatic venous outflow disease</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>57</td>
</tr>
</tbody>
</table>

1 Adapted from reference 52.

HISTOPATHOLOGY

Much of the confusion in earlier descriptions of ICC probably arose from differing liver biopsy criteria used by different workers (21-23). The striking orcein-rhodanine staining representing copper is seen consistently and easily in a simplified histologic diagnosis of ICC (Figure 1) (18). The two most discriminatory features of ICC now recognized are widespread, coarse, dark-brown orcein staining and intralobular pericellular fibrosis (24). Hepatocytic necrosis (seen in 97% of cases) and hyaline (seen in 66% of cases) are also diagnostic though late features. Portal fibrosis, inflammation, and disruption of the limiting plate are seen in most cases, but are seen also in other liver disorders and hence are not of discriminatory value. Parenchymal fat is usually absent and cholestasis is a late feature (11). Conditions that need careful differentiation on histologic grounds are chronic active (aggressive) hepatitis, which may show almost all features of ICC except the orcein staining and which, if present, is sparse and perilobar only, and inactive micronodular cirrhosis (cryptogenic cirrhosis), which may in fact be etiologically related to ICC (24).

COPPER IN INDIAN CHILDHOOD CIRRHOSIS

As seen in Table 2, hepatic copper as measured by atomic absorption spectroscopy is grossly raised in ICC. Hepatic copper is also modestly increased in neonates (physiologic), obstructive jaundice, and chronic active hepatitis (6, 8, 19). However, values > 12.6 μmol/g (800 μg/g dry wt) clearly distinguish ICC from other liver disorders in the ICC age group except Wilson disease, which is differentiated from ICC in several other ways (25) (Table 3).

Histologically, the copper in ICC is easily demonstrated by orcein (which stains copper-associated protein) and rhodanine (which stains copper itself). Ultrastructurally, this copper is not only concentrated in lysosomes (as dense aggregates of copper-sulfur) but also scattered widely in the cytosome (26, 27). Increased copper concentrations have been shown regularly in serum, hair, and urine (8, 28). Importantly, however, concentrations of ceruloplasmin are normal or raised in ICC compared with the characteristic lowering of ceruloplasmin in Wilson disease (29). These features are now used in the noninvasive diagnosis of ICC (27, 30) (Table 4).

WHERE DOES THIS MASSIVE AMOUNT OF COPPER COME FROM?

The increased copper in ICC may be secondary to liver damage and impaired biliary copper excretion (31, 32), may be the result of an inherited disorder of copper metabolism akin to Wilson disease (33), or may simply be the result of excessive copper intake. No studies to date have really substantiated the first two suggestions in typical ICC in India. However, many epidemiologic studies and feeding histories strongly support the copper-ingestion theory (3, 34-36). Copper and brass (an alloy of 70% copper and 30% zinc) vessels have been used extensively in India, especially by traditional Hindu families. Experimentally, boiling and storing milk in unlined brass vessels raises its copper concentration more than 60 times—a gross copper contamination (Table 5). Water takes up copper less avidly—storing water in a copper vessel raises its modest copper concentration only about six times (34). Ultracentrifugation and chromatography studies have shown that copper binds predominantly to casein, which is an exclusive constitu-

TABLE 2
Hepatic copper concentrations in Indian childhood cirrhosis (ICC) and other chronic liver diseases1

<table>
<thead>
<tr>
<th>Hepatic copper</th>
<th>μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>274 ± 240 (83-612)</td>
</tr>
<tr>
<td>0–1 mo (n = 15)</td>
<td>42 ± 24 (21-93)</td>
</tr>
<tr>
<td>6–12 mo (n = 5)</td>
<td>45 ± 35 (33-118)</td>
</tr>
<tr>
<td>ICC (n = 59)</td>
<td>1678 ± 694 (787-6654)</td>
</tr>
<tr>
<td>ICC siblings (n = 25)</td>
<td>648 ± 363 (62-1164)</td>
</tr>
<tr>
<td>Biliary atresia (n = 17)</td>
<td>286 ± 232 (121-144)</td>
</tr>
<tr>
<td>Chronic active hepatitis (n = 21)</td>
<td>166 ± 132 (11-302)</td>
</tr>
<tr>
<td>Wilson disease (n = 13)</td>
<td>837 ± 527 (311-1665)</td>
</tr>
<tr>
<td>Other cirrhosis (n = 18)</td>
<td>221 ± 210 (21-635)</td>
</tr>
<tr>
<td>Other diseases (n = 14)</td>
<td>108 ± 170 (17-436)</td>
</tr>
</tbody>
</table>

1 x ± SD; range in parentheses. Adapted from reference 19.

FIGURE 1. Characteristic orcein staining granules in Indian childhood cirrhosis (magnification × 400).
TABLE 3

<table>
<thead>
<tr>
<th>Characteristics of Wilson disease and Indian childhood cirrhosis (ICC): two pediatric copper storage disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson disease</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Family history</td>
</tr>
<tr>
<td>Presentation</td>
</tr>
<tr>
<td>Hepatic copper (µg/g)</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/L)</td>
</tr>
<tr>
<td>Histology</td>
</tr>
<tr>
<td>Orecin staining</td>
</tr>
<tr>
<td>Etiology</td>
</tr>
<tr>
<td>D-Penicillamine</td>
</tr>
</tbody>
</table>

Table: Siblings of Children with Indian Childhood Cirrhosis

Previous studies showed the clinical and histologic evolution of asymptomatic siblings into children with full-fledged ICC (38-40). Our studies in siblings with hepatomegaly showed a modest increase in hepatic copper concentrations but only minor histologic changes such as nonfatty ballooning of groups of hepatocytes and focal infiltration (41). None of these siblings (except one) progressed to ICC and subsequent biopsies showed reductions in copper deposition. The only intervention was a warning against the use of copper or brass for milk formulas. This suggests that there is a variable period of copper accumulation that is probably harmless and reversible, before the as yet unknown second factor sets off liver injury and resultant ICC (27).

Animal Studies

Feeding copper-contaminated milk caused modest hepatic accumulation of copper but not typical ICC in experimental lambs. In fact, the lambs died of hemolytic anemia due to a sudden release of copper into the circulation. Longer periods and larger doses of copper were required in experimental rats to cause hepatic copper accumulation (but not typical ICC) (42). Although large species differences make extrapolation to humans unwise (43), these experiments suggest that gross hepatic copper accumulation can occur because of copper-contaminated feeds. The question of what sets off damage leading to typical ICC in a copper-loaded liver remains unanswered.

Is There a Second Insult in Indian Childhood Cirrhosis?

Viral markers of hepatitis A and B, cytomegalovirus, and Epstein Barr virus in patients with ICC were not found to be significantly different from those in age-matched control subjects (44). Immunologic abnormalities often described in ICC, such as autoantibodies, low complement C4 concentrations, and circulating immune complexes, were found to be nonspecific and secondary as in other chronic liver disorders (11, 45).

TABLE 4

Table: Copper concentrations in Indian childhood cirrhosis (ICC) and other chronic liver diseases (CLDs)

<table>
<thead>
<tr>
<th>ICC</th>
<th>Other CLDs</th>
<th>Wilson disease</th>
<th>Reference (normal siblings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum copper (µmol/L)</td>
<td>22.0 ± 6.3</td>
<td>20.5 ± 11.0</td>
<td>12.6 ± 6.3 15.7-23.6</td>
</tr>
<tr>
<td>Serum ceruloplasmin (mg/L)</td>
<td>340 ± 170</td>
<td>590 ± 30</td>
<td>64 ± 64  &lt; 160</td>
</tr>
<tr>
<td>Ratio of urinary copper to creatinine (log10 µg/g)</td>
<td>4.1 ± 0.6</td>
<td>2.5 ± 0.9</td>
<td>3.2 ± 4.3  &lt; 2.0</td>
</tr>
</tbody>
</table>

1 Adapted from reference 34.
2 Cow, buffalo, and goat milk give similar results.
3 Breast milk (n = 12): 1.9-5.5 µmol/L.
Other toxins suggested in the past as causative agents, but largely discarded because of negative results, are arsenic, cadmium, and aflatoxins (27). Pyrrolizidine alkaloids, though known to accentuate copper toxicity in animals, could not be validated in field studies of ICC (46). Sophisticated collagen studies showed increased markers of fibrogenesis in ICC such as type III procollagen, 7S domain type IV collagen, and laminin. Primary abnormality of collagen metabolism, however, could not be implicated (47). Abnormal metallothionein metabolism, suggested by recent studies with cultured fibroblasts from an American child with ICC, could not be substantiated by similar studies of Indian children with ICC (48, 49). A strong genetic component of ICC has always been suspected because of familial occurrence and high consanguinity (50). From the present evidence therefore, it is still uncertain whether the second factor is a genetic predisposition, another environmental insult, or simply continued copper ingestion. However, because ICC is fast disappearing, searching for the second factor now appears a fairly futile exercise.

PREVENTION OF INDIAN CHILDHOOD CIRRHOSIS

Until the 1970s, ICC was thought to be the fourth most common cause of death in preschool children in India (51; oral communication, ICMR Workshop on ICC, Chandigarh, 1972). In 1981–1982, ICC accounted for 50% of all chronic liver disease, 5% of all pediatric admissions, and 10% of ward mortality at the KEM Hospital in Pune (20). Today ICC is a rarity in India, with only an occasional case reported annually even from large medical centers (Table 6) (52).

The copper-ingestion hypothesis of ICC suggested the remarkable possibility that a fatal liver disorder could be eradicated through a simple message of health education. This was demonstrated in Pune through an extensive interventional study (the population of Pune is 4.1 million in an area 15 642 km²) (12). A massive campaign of health education involving government and nongovernment health agencies (district health officials, multipurpose workers, Anganwadi workers, dairy societies, and adult education workers) was carried out in the entire Pune district from 1984 to 1987. Systematic surveys showed that the campaign reduced the use of brass vessels in the Pune district from 13% to 4%. This was associated with a significant fall in the number of cases of ICC from the Pune District seen at KEM Hospital. During this period no such interventions reached the Chandigarh area and the number of children with ICC presenting to the Postgraduate Institute in Chandigarh did not change. Interestingly, despite no active intervention in the neighboring Ahmednagar District (120 km from Pune), brass usage “spontaneously” decreased from 78% to 32%, which was associated with a significant fall in ICC in this area. Although spontaneous reduction in use of brass vessels has not been documented in the rest of India, it appears to be the most plausible reason for the drastic reduction in numbers of ICC cases all over the country. Dairy development and government milk schemes have led to replacement of the milkman’s (Dudhwalla’s) brass vessels with aluminum cansisters and glass or plastic bottles for milk supply. With urbanization and smaller families now the norm, household brass vessels have been put away in favor of cheaper and harder stainless steel or aluminum containers.

TABLE 6
Annual incidence of Indian childhood cirrhosis (ICC) and chronic liver disease (CLD) in major pediatric centers in India (1992–1993)

<table>
<thead>
<tr>
<th>Institute, city</th>
<th>All admissions</th>
<th>All CLD</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institute of Child Health, Madras⁷</td>
<td>28 500</td>
<td>254</td>
<td>3</td>
</tr>
<tr>
<td>National Medical College, Calcutta²</td>
<td>2840</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>LPJN Hospital (Maulana Azad), New Delhi³</td>
<td>13 950</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Sanjay Gandhi Postgraduate Institute, Lucknow⁴</td>
<td>—</td>
<td>48</td>
<td>1</td>
</tr>
<tr>
<td>Postgraduate Institute of Medical Education and Research, Chandigarh⁵</td>
<td>—</td>
<td>52</td>
<td>3</td>
</tr>
</tbody>
</table>

¹ From VS Sankaranarayanan.
² From Bijon Chakraborty.
³ From SK Mittal.
⁴ From SK Yachha.
⁵ From BNS Wallia.

SURVIVORS OF INDIAN CHILDHOOD CIRRHOSIS

Therapeutic trials of the copper chelator D-penicillamine for ICC treatment have shown a remission in up to 65% of patients in the early (preicteric) stage of disease (53). Remission is associated with clinical recovery, reduction in hepatic copper to normal concentrations, and striking histologic reversal of cirrhosis within a couple of years of therapy (54–56). The sequence of reversal usually seen is (Figure 2A–D) 1) pseudolobules with activity, 2) inactive micronodular cirrhosis with healthy cells, and 3) thinning and breaking down of septa, leading to virtually normal histology. Twenty-nine such survivors of ICC (followed up for 5–12 y) have continued to do well despite the withdrawal of D-penicillamine after 3–6 y of ther-

FIGURE 2. (A) Characteristic histologic features of Indian childhood cirrhosis (ICC) at presentation: pericellular fibrosis, hepatocytic necrosis, and inflammatory cell infiltrate. Magnification × 400. (B) ICC after 6 mo of therapy with D-penicillamine, showing pseudolobule formation and reduced hepatocellular injury. Magnification × 400. (C) Stage of inactive micronodular cirrhosis with thin septae and minimal parenchymal damage, 1 y after therapy. Magnification × 40. (D) Virtually normal biopsy showing minimal fibrosis (reversal of cirrhosis). Magnification × 100. All panels, hematoxylin-eosin staining.
The continued well-being of ICC survivors without d-penicillamine and the disappearance of ICC coincident with a decrease in the use of brass vessels for feeding strengthen the evidence that copper accumulation in ICC is an acquired phenomenon, rather than an inborn error of copper metabolism.

INDIAN CHILDHOOD CIRRHOSIS IN WESTERN COUNTRIES

Recent reports of ICC-like cirrhosis with raised hepatic copper concentrations in Western countries have raised doubts yet again about the copper-ingestion theory of ICC (13–16). Although copper-contaminated water (copper pipes and water with a low pH) has been incriminated in a few cases, the amount of copper ingested does not match that in ICC in India. The sometimes familial occurrence, and also a history of consanguinity, suggests a strong genetic component of this disease. Recent studies in molecular biology have shown extreme heterogeneity in the Wilson disease gene, suggesting that some disruptions or mutations could be responsible for very early onset disease akin to ICC (57, 58). Different mechanisms, environmental or genetic (or combinations thereof), can lead to the same end-stage liver disease, giving rise to the concept of idiopathic copper toxicity or copper-associated childhood cirrhosis. If so, ICC could well represent a specific form of idiopathic copper toxicity or copper-associated childhood cirrhosis that requires high environmental copper ingestion for its full expression (59). The change in traditional feeding vessels explains the virtual disappearance of ICC in India. It also means that an odd case of ICC without convincing evidence of copper ingestion (often labeled in the past as atypical ICC) will continue to be seen, but the numbers hopefully will be too small to be considered a health problem of children in India.

We gratefully acknowledge the following persons for invaluable help in carrying out this entire project: MS Tanner for inspiring the study; Avinash Pradhan for histopathologic studies; SS Dodwad, District Health Officer who monitored the District Health Education Campaign; SG Ramdas, Assistant Director of Health Services, who provided the Health Education materials; and the Research Officers and Medical Social Workers, who diligently carried out the research for 15 y.

REFERENCES

44. Tanner MS, Flower AJE, Bhave SA, Pandit AN. Does Indian childhood cirrhosis result from viral infection in a copper-laden liver. Gut 1982;23:A922–3.