

Hepatic Copper Accumulation in Primary Biliary Cirrhosis

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Hepatic copper accumulation is a regular feature of primary biliary cirrhosis (PBC). The levels are directly related to the clinical stage of the disease. Since the copper values in PBC are comparable to Wilson's disease, there is the potential for copper toxicity, although this is speculative since the two diseases differ in the binding, distribution, and intracellular localization of the copper. The involvement of copper toxicity in the progression of PBC is supported by the observation that the highest values occur in association with the hepatic failure that occurs in the advanced stage.

Corticosteroid therapy appears to decrease hepatic copper levels in PBC. Although this therapy does not invariably lower the hepatic Cu content in patients with PBC, it does so in many individuals. Therapeutic trials with d-penicillamine are in progress. When results are available they will guide us in the management of individual patients with PBC. In the meantime, dietary copper should be restricted as is done in management of Wilson's disease.

Since copper homeostasis is largely maintained by biliary excretion, any process which results in chronic cholestasis may be associated with hepatic copper accumulation [1,2]. Increased hepatic copper levels have been observed in primary biliary cirrhosis (PBC) [2-5] and prolonged bile duct obstruction [5], with the levels in PBC frequently being in the range associated with untreated Wilson's disease [6,7]. The hepatic damage in Wilson's disease is attributed to excess copper although the pathogenesis of the tissue injury has not been defined [8]. Since the pathogenesis of hepatic damage in PBC remains unknown, it is possible that copper toxicity contributes to the progression of the liver disease. This suggestion is supported by comparable hepatic copper concentrations in both diseases and has resulted in the initiation of therapeutic trials with d-penicillamine in PBC [9-11].

This study was initiated to determine the regularity of copper accumulation in PBC and document the relationship between the copper levels and the clinical stage of the disease.

METHODS

The diagnosis of PBC was established in 56 patients by the usual criteria of a compatible clinical picture, presence of antimitochondrial antibody, and appropriate morphological changes [12,13]. The patients were classified as asymptomatic (17), active (32), or advanced (7), according to their clinical status. The patients in the

asymptomatic group were recognized because of abnormalities in the liver studies, particularly the alkaline phosphatase, evidence of a lipid disorder or hepatomegaly. All patients in this group had a serum bilirubin of less than 1.2 mg/dl. Patients in the *active* group had symptoms referable to chronic liver disease. These patients were generally referred for evaluation because of active liver disease, jaundice, or pruritis. In this group of 32 patients, there were 12 who had been treated for periods longer than 3 months with corticosteroids in varying dosages. The patients in the *advanced* group were jaundiced with severe cholestasis and evidence of progressive liver failure. Five of the seven patients in this category died during the period of observation.

Liver biopsy specimens were handled to prevent trace metal contamination. The tissue was freeze-dried and the dry weight determined. The dried tissue was wet-ashed with nitric-sulfuric acid, diluted and analyzed for copper by atomic absorption spectrophotometry utilizing peak-retrieval circuitry [14]. Hepatic copper values are expressed as $\mu\text{g/g}$ liver (dry weight). Based on the literature and analysis in patients with a variety of diseases, $50 \mu\text{g/g}$ or less was considered to be normal [5,8].

RESULTS

Figure 1 shows the individual hepatic copper values for each of the 56 patients in relationship to the clinical stage. In the *asymptomatic* group, hepatic copper was $114 \pm 81 \mu\text{g/g}$ (mean \pm S.D.) with a range of $32\text{--}298 \mu\text{g/g}$. Thirteen of the 17 values were abnormal, although only one exceeded $250 \mu\text{g/g}$, the level which might be confused with Wilson's disease [6,7]. In the *active* stage, the hepatic copper level was $342 \pm 292 \mu\text{g/g}$ (mean \pm S.D.) with a range of $13\text{--}1,249 \mu\text{g/g}$. Twenty-seven of 32

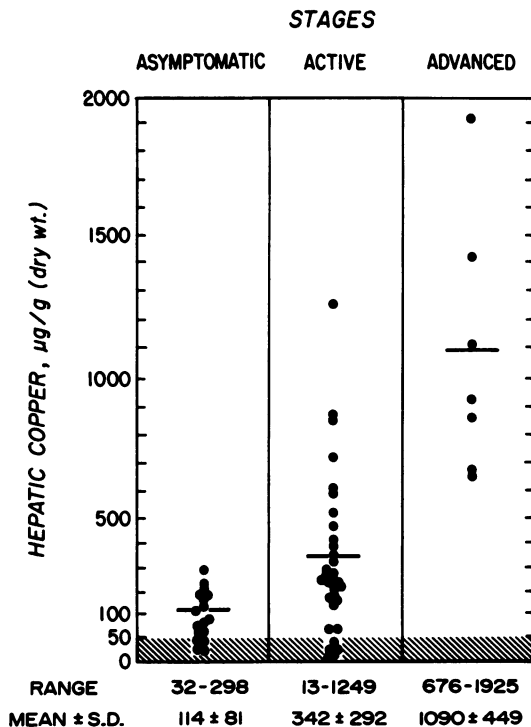


FIG. 1. Hepatic copper levels, $\mu\text{g/g}$ (dry weight), in asymptomatic, active and advanced stages of primary biliary cirrhosis. Shaded area, normal range; horizontal lines, mean values.

values were abnormal and 16 of 32 exceeded $250 \mu\text{g/g}$. All values were markedly abnormal in the *advanced* stage, $1,090 \pm 449 \mu\text{g/g}$ (mean \pm S.D.) with a range of $676\text{--}1,925 \mu\text{g/g}$. Because of the distribution, log transformations were performed on all data. There was a highly significant difference between groups when evaluated by a one-way analysis of variance. Newman-Keuls tests for all possible pair-wise comparisons showed that the groups are significantly ($p = .01$) different from each other.

When patients in the active stage were evaluated for corticosteroid therapy, 12 were found to have received varying dosages for periods greater than three months. Figure 2 compares the results in the steroid group with the non-steroid group. The hepatic copper level on the steroid group was $240 \pm 251 \mu\text{g/g}$ (mean \pm S.D.) with a range of $13\text{--}790 \mu\text{g/g}$. Four values were in the normal range and two were near normal ($69 \mu\text{g/g}$). In the non-steroid group the level was $403 \pm 304 \mu\text{g/g}$ (mean \pm S.D.) and a range of $32\text{--}1,249 \mu\text{g/g}$. The one normal value of $32 \mu\text{g/g}$ occurred in a patient with mild symptoms and might well have been included in the asymptomatic group. Analysis by Student *t*-test showed a significant ($p = .02$) difference between the mean hepatic copper levels in the two groups. When the number of values in the normal range in the steroid and non-steroid groups are compared (using Fisher's exact probability test) significantly more are in the steroid group ($p = .05$). In the steroid group, 4 of 12 values were normal while only 1 of 20 was normal in the non-steroid group. The analysis was biased against a difference being related to corticosteroid therapy since values of 342 and $790 \mu\text{g/g}$ were included in the steroid group although this therapy had been terminated one and two years before evaluation.

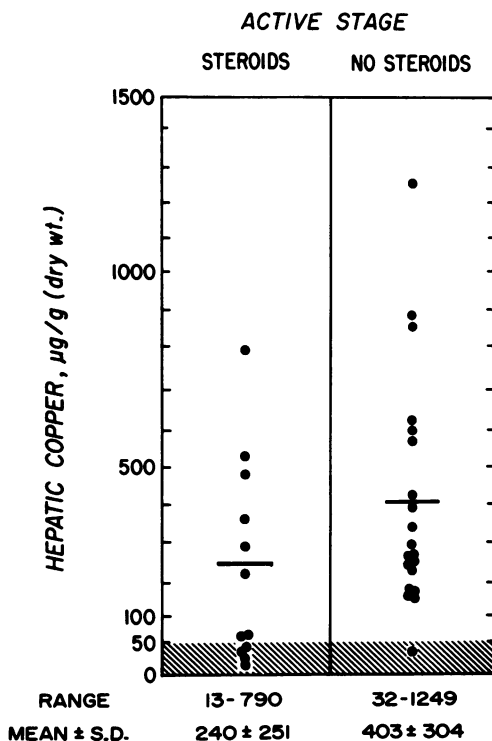


FIG. 2. Hepatic copper levels, $\mu\text{g/g}$ (dry weight), during active stage of primary biliary cirrhosis. Left column, 12 patients who received corticosteroids before evaluation; right column, 20 patients who did not receive corticosteroids. Shaded area, normal range; horizontal lines, mean values.

During the study nine patients had repeat biopsies after receiving continuous or intermittent corticosteroid therapy for intervals of 12–46 months. Six of the nine showed a decrease in hepatic copper. One patient showed an increase from 60 to 492 $\mu\text{g/g}$ and progression from asymptomatic to an active stage over four years. This patient was found to be using a multivitamin preparation which contained 2 mg of copper sulfate on a regular basis during the four-year period. This may have contributed to the increase in hepatic copper, and, possibly, to the progression of the disease process. Five of six patients who did not receive steroids showed a mild increase in hepatic copper during 11 to 49 months of observation. Because these observations were not controlled they were not analyzed further.

DISCUSSION

The current study shows that there is a clear relationship between the levels of hepatic copper and the stage of the disease. Hepatic copper accumulation is a regular feature of PBC at the active or advanced stage. The hepatic copper levels are directly related to the severity of cholestasis although they may also be a reflection of the duration of the disease process.

An unanswered question is whether copper accumulation is involved in the pathogenesis of PBC, or whether it is merely a result of prolonged cholestasis. Although it is attractive to speculate that copper toxicity is responsible for tissue damage in Wilson's disease and PBC, there are some significant differences in the localization and binding of the copper which limit the attractiveness of this reasoning. The excess copper in Wilson's disease appears to be associated mainly with the particulate fraction of the subcellular compartments, predominately the lysosomes [6,15], whereas the excess copper is in the supernatant fraction in PBC [16]. In addition, the copper-binding protein demonstrated in the periportal hepatocytes of patients with prolonged cholestasis [17], particularly the form observed in PBC [18] appears to differ from that observed in Wilson's disease. Therefore, although the excess copper in the hepatocytes may contribute to progressive liver damage, the mechanism of injury may differ from that in Wilson's disease since the site of deposition, distribution, and binding are different.

Since the mean hepatic copper level was only 114 $\mu\text{g/g}$ at the asymptomatic stage, marked copper accumulation is not associated with the initial inflammatory process. This contrasts with Wilson's disease where the highest hepatic copper levels occur during the asymptomatic or preclinical period [6,7,8]. The extremely elevated levels noted during the advanced stage of PBC, mean value of 1,090 $\mu\text{g/g}$, were associated with hepatic failure. Six of the seven patients in this group died as a result of the disease. This relationship between the hepatic copper levels and the stages of the disease supports the involvement of copper toxicity in the progression of PBC rather than the initiation of the disease, as appears to be the case in Wilson's disease.

Between the asymptomatic and the advanced stages were patients judged to be in an active phase of the disease. In this group, the values ranged from normal to 1,249 $\mu\text{g/g}$ with a mean of 342 $\mu\text{g/g}$. Initially, all patients studied in this phase of the disease had elevated hepatic copper levels but, as the series was extended, several were observed to be normal or near normal. Therefore, it appears that PBC can be symptomatic without significant elevation of hepatic copper. Analysis of these patients disclosed that 4 of 5 with normal levels had been treated previously with corticosteroids. Corticosteroid therapy has been widely used for chronic active hepatitis and, because of confusion with regard to the diagnosis, is occasionally

prescribed for the patient with PBC. Corticosteroid therapy has been considered to be contraindicated in PBC because of potentiation of bone disease [12] and, in one published series, the absence of a response was used to differentiate PBC from chronic active hepatitis [19]. Nevertheless, some patients with established PBC do appear to show clinical improvement with this therapy, although the benefit may not be great enough to justify long-term treatment. In the current study those patients who were normal or near normal during the active stage of the disease were very likely to have been treated with corticosteroids. Six of nine patients who received steroid therapy during this study showed a mild decrease in hepatic copper. These observations suggest that corticosteroid therapy facilitates the biliary excretion of copper perhaps by decreasing the inflammatory process, though the mechanism has not been demonstrated. This response may occur in the absence of significant clinical improvement and suggests that corticosteroids have an effect in PBC that has not been recognized previously.

Controlled studies are in progress to evaluate therapy with d-penicillamine in PBC because of the abnormal hepatic copper levels [9,11]. Because of the chronicity of PBC it may not be possible to determine the beneficial effect of this therapy for several years. Any beneficial effect may be difficult to document unless the patients are observed for prolonged periods since it is possible that treatment prevents or delays the onset of hepatic failure, rather than reversing the process present at the time of initiation of therapy. If copper removal were the only beneficial effect of d-penicillamine in PBC, one would not expect much change in patients in the asymptomatic phase of the disease. It should be remembered that d-penicillamine also has the potential for altering immune responses [20] and retarding fibrogenesis [21,22], factors which appear to be operative in the pathogenesis of PBC. Since therapy with d-penicillamine is frequently associated with side-effects, it seems prudent to await results of the controlled studies before initiating treatment in individual patients. Until such information is available, it may be appropriate to restrict copper intake as is done for therapy of Wilson's disease.

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