Liver disease in transfusion dependent thalassaemia major

C K Li, K W Chik, C W K Lam, K F To, S C H Yu, V Lee, M M K Shing, A Y K Cheung, P M P Yuen

Aims: To study the prevalence and severity of liver diseases of transfusion dependent thalassaemia major patients, and correlate the histological and biochemical changes of iron overload in liver with the peripheral blood markers.

Method: Liver biopsy was performed to assess the histological changes and liver iron content (LIC).

Results: One hundred patients were evaluated (median age 11.7 years, range 1.5–27). A total of 81 liver biopsies were performed in 73 patients; 43 samples were analysed for LIC. Grade 3–4 haemosiderosis and hepatic fibrosis was found in 44% and 30% of patients respectively; both were significantly associated with higher serum ferritin, liver enzymes, and LIC. Very high LIC (>15 mg/g dry weight) was present in 16.3% of patients.

Conclusion: Severe haemosiderosis and hepatic fibrosis were common in patients with thalassaemia major despite the use of chelation therapy. Liver biopsy provided information on fibrosis and LIC which could not be accurately predicted from peripheral blood markers.

Thalassaemia major (TM) is the commonest hereditary form of transfusion dependent anaemia in Hong Kong and about 300 patients currently receive regular blood transfusion treatment. With regular transfusion and iron chelation, most TM patients now survive beyond the third decade. Although cardiomyopathy is still the leading cause of death in this group of patients, liver disease is becoming a more important cause of mortality. Cirrhosis and hepatocellular carcinoma may develop as result of chronic hepatitis and/or severe iron overload. Iron chelation with desferrioxamine can reduce excessive body iron, but efficacy is limited by poor compliance and dose related toxicity. The prevalence of cirrhosis is reported to be 10–40% and the prevalence of liver fibrosis is about 40–80%, but these data are over 10 years old. The prevalence of liver disease in TM patients in recent years is not known, and there are no such data from Oriental populations. Since 1980, patients have been given desferrioxamine at a younger age, and so iron overload and its related complications may be less severe. The screening of blood products for hepatitis C antibody has reduced the risk of hepatitis. This study examined the prevalence and severity of liver disease in Chinese TM patients treated in a single institution. The correlation between histological and biochemical markers of iron overload was also examined.

PATIENTS AND METHODS

This was a retrospective study of TM patients treated at the Department of Paediatrics, Prince of Wales Hospital. One hundred TM patients (48 males and 52 females) were included. Forty four had undergone allogeneic bone marrow transplantation (BMT) and 56 patients were receiving regular blood transfusions. Median age at diagnosis was 0.7 years, with 75% less than 1 year. Blood transfusions were administered at 3–4 weekly intervals, to maintain a pretransfusion haemoglobin above 95 g/l. Desferrioxamine was given by subcutaneous infusion over 8–10 hours (20–50 mg/kg), generally 5–6 times weekly. Median age of starting chelation was 3.7 years (range 1.1–18 years). The patients had received chelation therapy for a median of 7.7 years (range 1 month to 22 years). They had received regular transfusions for a median of 10.6 years (range 1 month to 25 years). At time of evaluation, the median age was 11.7 years (range 1.5–27 years) (table 1). Two patients were positive for hepatitis B surface antigen. Eight were positive for hepatitis C antibody and for hepatitis C RNA.

Liver biopsy

Liver biopsy had been performed in 37 patients prior to BMT between 1992 and 1999. A total of 36 transfusion dependent (TD) patients underwent liver biopsy to assess their liver iron content (LIC). This LIC study was approved by the institution’s ethical committee. Informed consent for liver biopsy was obtained from each patient. A total of 81 biopsies were performed on 73 patients. The biopsy specimens were obtained from the right lobe of the liver under ultrasound guidance using a semiautomated 16 gauge Tru-cut style needle (Tenco, Bauer Medical International, Dominican Republic). A core of tissue was sent for LIC analysis by graphitite furnace atomic absorption.

Histological study

A core of liver tissue was evaluated histologically and graded semiquantitively for iron storage by a single pathologist. Liver sections were stained with standard haematoxylin and eosin. Iron was shown using the Perls' stain procedure. Grading was based on the method of Scheuler modified by Rowe, which classified parenchymal and mesenchymal haemosiderin deposition as follows: grade 0, no haemosiderin; grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, heavy. The presence or absence of fibrosis was recorded. The liver was assessed for evidence of hepatitis.

Biochemical markers

Serum ferritin was measured at six monthly intervals, and the mean serum ferritin in the year before evaluation was recorded. Serum alanine aminotransferase (ALT) levels were

Abbreviations: ALT, alanine aminotransferase; BMT, bone marrow transplantation; LIC, liver iron content; TD, transfusion dependent; TM, thalassaemia major
routinely measured prior to monthly transfusion, and levels in
the two year period before this evaluation were recorded.

Statistics
Continuous variables were expressed as mean and standard
deviation. Comparison of factors potentially associated with
haemosiderosis, fibrosis, and elevated serum transaminase
was performed using Student’s t test or analysis of variance as
appropriate. These variables included sex, duration of transfu-
sion and chelation, age, serum ferritin, LIC, and hepatitis sta-
tus. The possible associations between LIC and serum ferritin,
and haemosiderosis deposition were explored using Pearson’s
correlation coefficient or Fisher’s exact test. Multivariate
analysis by stepwise regression for factors predicting haemo-
siderosis, hepatic fibrosis, and liver transaminase elevation
were performed. The level of significance was set at 0.05 in the
analyses and all the statistical testing was two sided.

RESULTS
Histology
All the liver specimens showed at least grade 1 haemosidero-
sis; grade 3 and 4 occurred in 26% and 18% of the samples
respectively (table 2). Serum ferritin, ALT, and LIC were
significantly different among the four grades of haemosidero-
sis (p < 0.001, p = 0.001, and p < 0.001 respectively). The
differences remained significant when grade I and II
combined were compared grade III and IV. There was no sig-
nificant difference in age at evaluation, or in the duration of
transfusion and chelation. Multivariate analysis revealed
serum ferritin as the most significant predictor of moderate to
severe haemosiderosis (p = 0.003). However, when multivari-
ate analysis was applied to the 42 in whom LIC was available,
LIC appeared to be an even better predictor of moderate to
severe haemosiderosis (p < 0.001).

Features of hepatitis were reported in seven of the eight
patients positive for hepatitis C, but none of these had features
of cirrhosis. The two patients positive for hepatitis B surface
antigen did not have evidence of hepatitis. Hepatic fibrosis was
present in 22 cases and these patients had significantly higher
concentrations of serum ferritin, serum ALT, and LIC (table 2).
Hepatic fibrosis was more common in those with hepatitis C
(p = 0.008). Multivariate analysis revealed serum ferritin as
the most significant predictor of hepatic fibrosis.

Table 1  Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Biopsy (n=73)</th>
<th>Non-biopsy (n=27)</th>
<th>Total (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (y)</td>
<td>1.1 (1.6)</td>
<td>1.5 (1.8)</td>
<td>1.2 (1.6)</td>
</tr>
<tr>
<td>(Range)</td>
<td>(0–11.2)</td>
<td>(0–8.3)</td>
<td>(0–11.2)</td>
</tr>
<tr>
<td>Age started iron chelation (y)</td>
<td>4.6 (2.7)</td>
<td>4.4 (3.6)</td>
<td>4.5 (2.9)</td>
</tr>
<tr>
<td>(Range)</td>
<td>(1.1–18)</td>
<td>(1.5–16)</td>
<td>(1.1–18)</td>
</tr>
<tr>
<td>Age at evaluation (y)</td>
<td>11.7 (4.5)</td>
<td>10.9 (7.4)</td>
<td>11.5 (5.4)</td>
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<tr>
<td>(Range)</td>
<td>(1.5–22.5)</td>
<td>(2.7–27)</td>
<td>(1.5–27)</td>
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<tr>
<td>Duration of transfusion (y)</td>
<td>10.7 (4.9)</td>
<td>9.4 (6.8)</td>
<td>10.3 (5.5)</td>
</tr>
<tr>
<td>(Range)</td>
<td>(0.1–21.5)</td>
<td>(0.7–25)</td>
<td>(0.1–25)</td>
</tr>
<tr>
<td>Duration of chelation (y)</td>
<td>7.4 (3.8)</td>
<td>6.9 (5.3)</td>
<td>7.3 (4.3)</td>
</tr>
<tr>
<td>(Range)</td>
<td>(0.1–19.5)</td>
<td>(0.5–22)</td>
<td>(0.1–22)</td>
</tr>
<tr>
<td>Average ferritin (pmol/l)</td>
<td>5205 (3634)</td>
<td>4727 (2145)</td>
<td>5078 (3301)</td>
</tr>
<tr>
<td>(Range)</td>
<td>(970–21000)</td>
<td>(1395–10449)</td>
<td>(970–21000)</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>38 (45)</td>
<td>40 (39)</td>
<td>38 (43)</td>
</tr>
<tr>
<td>(Range)</td>
<td>(9–299)</td>
<td>(1–186)</td>
<td>(1–299)</td>
</tr>
<tr>
<td>LIC* (mg/g dry weight)</td>
<td>9.6 (8.8)</td>
<td></td>
<td></td>
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<tr>
<td>(Range)</td>
<td>(0.8–41.8)</td>
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</table>

Results expressed as mean (SD).

Table 2  Histological changes of liver biopsy specimens and associated factors

<table>
<thead>
<tr>
<th></th>
<th>Serum ferritin (pmol/l)</th>
<th>ALT (IU/l)</th>
<th>LIC (mg/g)</th>
<th>Age (y)</th>
<th>Transfusion (y)</th>
<th>Chelation (y)</th>
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</thead>
<tbody>
<tr>
<td>Haemosiderosis</td>
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<tr>
<td>Grade I</td>
<td>17 (23%)</td>
<td>2981 (1256)</td>
<td>17 (5)</td>
<td>5.3 (3.0)</td>
<td>10.4 (3.0)</td>
<td>9.3 (3.1)</td>
</tr>
<tr>
<td>Grade II</td>
<td>24 (33%)</td>
<td>4463 (1814)</td>
<td>30 (28)</td>
<td>6.7 (3.8)</td>
<td>11.7 (4.8)</td>
<td>10.8 (4.8)</td>
</tr>
<tr>
<td>Grade III</td>
<td>19 (26%)</td>
<td>6151 (4215)</td>
<td>38 (28)</td>
<td>9.9 (5.4)</td>
<td>13.2 (4.4)</td>
<td>12.8 (4.4)</td>
</tr>
<tr>
<td>Grade IV</td>
<td>13 (18%)</td>
<td>5205 (3634)</td>
<td>78 (82)</td>
<td>22.7 (14.3)</td>
<td>11.4 (5.5)</td>
<td>9.2 (6.8)</td>
</tr>
<tr>
<td>p=0.001</td>
<td></td>
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<tr>
<td>Grade I/II</td>
<td>41 (56%)</td>
<td>3848 (1752)</td>
<td>24 (22)</td>
<td>6.2 (3.5)</td>
<td>11.1 (4.1)</td>
<td>10.2 (4.2)</td>
</tr>
<tr>
<td>Grade III/IV</td>
<td>32 (44%)</td>
<td>6999 (4617)</td>
<td>54 (59)</td>
<td>15.5 (11.8)</td>
<td>12.5 (4.9)</td>
<td>11.3 (6.7)</td>
</tr>
<tr>
<td>p=0.001</td>
<td></td>
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<tr>
<td>Hepatic fibrosis</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Present</td>
<td>22 (30%)</td>
<td>7717 (4193)</td>
<td>72 (67)</td>
<td>13.9 (11.8)</td>
<td>13 (4.8)</td>
<td>11.6 (5.7)</td>
</tr>
<tr>
<td>p=0.001</td>
<td></td>
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</tr>
<tr>
<td>Absent</td>
<td>51 (70%)</td>
<td>4170 (2824)</td>
<td>23 (16)</td>
<td>8.0 (6.9)</td>
<td>11.2 (4.3)</td>
<td>10.3 (4.5)</td>
</tr>
<tr>
<td>p=0.048</td>
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</table>

Results expressed as mean (SD).
Liver iron content

Forty three samples were analysed for LIC. The median LIC was 7.5 mg/g dry weight (range 0.8–41.8). Seven samples (16.3%) had an LIC over 15 mg/g, a concentration associated with a high risk for cardiac disease. The correlation coefficient for LIC and serum ferritin was 0.687 (p < 0.001) (fig 1). Considering those with a serum ferritin greater than 5500 pmol/l (2500 µg/l) (a concentration associated with increased mortality) the correlation was only 0.46 (p = 0.11). There were significant differences in LIC in association with the various grades of haemosiderosis (p < 0.001) (fig 2).

Liver enzyme evaluation

There was no correlation between serum ALT concentrations and age, duration of transfusion and chelation, or LIC. Serum ferritin and anti-HCV positivity were the only significant factors associated with increased ALT concentrations (both p < 0.001). Eighteen patients (18%) had persistent elevation of ALT and 10 (10%) had transient elevation. The transiently elevated group had significantly higher serum ferritin than the non-elevated group (p < 0.001).

DISCUSSION

Despite the use of prenatal screening in Hong Kong for more than 10 years, there are still two to five new cases of TM annually. These represent either failures of screening or immigrant children from mainland China. Though the number of reported deaths from liver disease in TM is small, severe haemosiderosis and hepatic fibrosis may lead to cirrhosis. Treatment is now available for hepatitis C infection. Thus, early and accurate diagnosis of liver disease followed by prompt intervention may prevent liver disease progression. In our study, we found a prevalence of 44% grade 3–4 haemosiderosis; this is similar to that reported approximately 10 years ago. This could reflect the rather unsatisfactory compliance rate with desferrioxamine therapy observed in many of our patients.

Desferrioxamine induced toxicity is relatively common in patients with less severe iron overload. Serum ferritin is an acute phase reactant and may be increased as a result of inflammatory disorders such as hepatitis and ascorbate status. Measurement of LIC is an alternative approach to the assessment of body iron content. This has been studied in both TM and hereditary haemochromatosis patients and is found to be a more accurate indicator of overload. The safety of liver biopsy has been shown in TM patients. The accurate assessment of body iron stores can help guide chelation therapy, and thus reduce desferrioxamine toxicity. Unfortunately, liver biopsy is an invasive procedure, and patients may not accept frequent biopsy as suggested by some authors. Identifying patients who are at high risk of developing fatal complications from iron overload is an important objective. Continuous central venous administration of desferrioxamine may postpone cardiac death.

Although there was a correlation between serum ferritin and LIC in this study, this was less reliable at ferritin concentrations above 5500 pmol/l. Patients with “low” serum ferritin can be monitored without biopsy but LIC should be measured in those with a high serum ferritin concentration. Low serum ferritin should alert the physician to the need for desferrioxamine for dose reduction in order to avoid toxicity.

Liver histological examination provides information about haemosiderosis and hepatic fibrosis. While LIC measurement may not be available in some centres, the grading of haemosiderosis provides a useful measure of iron overload as there was good correlation with LIC. Grade 4 haemosiderosis was always associated with an LIC of more than 10 mg/g dry weight, and such patients require intensive chelation therapy. The presence of hepatic fibrosis points to a risk of progression to cirrhosis. The pathogenesis of hepatic fibrosis in iron overload is still not well understood, although severe overload is a known risk factor for developing fibrosis. With effective iron chelation, hepatic fibrosis may be reversible. Hepatic fibrosis is also a known prognostic factor for patient survival following BMT, as reported previously.

In this study, we did not observe any patients with liver cirrhosis. The 30% prevalence of hepatic fibrosis was lower than that reported previously. In our study, only eight patients were positive for hepatitis C, a much lower prevalence than that reported in Mediterranean TM. This may be a result of the relatively young age of our patients and also the screening of blood products since the early 1990s. The hepatitis C positive patients have entered a trial of combination treatment with interferon and ribavirin.

In conclusion, liver disease is common in TM patients and severe haemochromatosis is still observed in 30–40% of patients despite the use of desferrioxamine chelation. Serum ferritin is useful to monitor treatment in patients who have not yet developed severe iron overload. However patients with high serum ferritin concentrations and those with hepatitis C should be evaluated for LIC, and for hepatic fibrosis and cirrhosis by liver biopsy. In centres where LIC assay is not available, histological grading of haemosiderosis is a useful alternative.

ACKNOWLEDGEMENT

We thank the Hong Kong Children’s Thalassaemia Foundation for financial support of the liver iron content study.
Liver disease in transfusion dependent thalassaemia major

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HIV therapy and folate antagonists in the first trimester can lead to birth defects, a UK study has found. With HIV increasing among women, more are taking antiretroviral therapy (ART) and other drugs with the potential for causing birth defects to control HIV associated conditions. No excess risk of birth defects arises from ART, according to the International Antiviral Pregnancy Agency, but no data exist for use with other extensively used drugs like folate antagonists.

Jungmann et al looked for links between use of ART and folate antagonists in the first trimester and birth defects in a retrospective case note study of mother-infant pairs. The mothers, known to be HIV positive beforehand, delivered at one of six inner London hospitals from May 1994 to June 1999.

Among 195 mother-infant pairs ART was used increasingly in the first trimester (0% to 28%, 1994 versus 1998). Forty four (23%) infants were exposed to other drugs in the first trimester. Nine infants (4.6%) had birth defects. Infants exposed to both ART and folate antagonists during the first trimester had seven times the risk of birth defects of those exposed to neither 23.1% (3/13) versus (4% (6/148); odds ratio 7.10, 95% confidence interval 1.5 to 34.2). None of 34 infants exposed to ART or folate antagonists alone in the first trimester had birth defects.

Excluding maternal characteristics as possible confounders, the authors concede their study’s other limitations. Still, they say, doctors should keep reviewing the need for prophylactic folate antagonists in women of childbearing age.

Sexually Transmitted Infections 2001;77:441–3.