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Short communication

# Introducing a new histologic scoring system for iron deposition in liver of thalassemic patients, compared with atomic absorption spectrometry

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ABSTRACT

Iron deposition in liver is a major finding in thalassemic patients and because of direct iron toxicity to liver it is associated with several consequences for example liver fibrosis. Liver biopsies from 63 patients were evaluated, 40 (63.5%) were male and 20 (36.5%) were female. The mean age of the patients was 8.01 ± 3.7 and the age range was from 1.8 to 15 years. Histologic grading and staging was performed for each case according to modified HAI (Hepatitis Activity Index) system. Iron scoring was performed according to Sindram & Marx and MTK1–3 scoring systems. The mean (SD) dry weight (dw) of liver specimens was 1.34 (0.11) mg (range 0.20 to 3.80 mg). The mean (SD) of hepatic iron concentration was 230.9 (121.2) μmol/g dry weight. The relationship between the variables HIC, HII (hepatic iron index) and all histological gradings of iron (S&M and MTK1–3) was very strong. The relationship between the HIC and staging by HAI method was good. Significant differences were identified between the mean HIC in scores 1&2 of all histological iron scorings (S&M and MTK1–3), but no significant differences identified between mean HIC in other adjacent scores in all histological iron scorings (S&M and MTK1, 2 and 3). New scoring system introduced by us in this study which considered size and density of iron granules as well as zone of iron deposition was very much the same as simple Sindram and Marx classification.


## 1. Introduction

Thalassemia major (TM) is the commonest hereditary form of transfusion dependent anemia in some areas of the world including Iran. Most patients currently receive regular blood transfusions (Mahjoub et al., 2004; Li et al., 2002). With regular transfusions and iron chelation, most TM patients now survive beyond the third decade (Borgna-Pignatti et al., 1998). Although cardiomyopathy is still the leading cause of death in this group of patients, liver disease is becoming a more important cause of mortality (Zurlo et al., 1989). 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 (Li et al., 2002).

Bone marrow transplantation is done in some countries such as Iran for these patients. Determination of liver iron load and grading and staging is part of patient assessment before bone marrow transplantation.

The direct measurement of hepatic iron overload is usually based upon two complementary methods: biochemical determination of liver iron concentration (LIC) and histological examination (HISTO). The biochemical assay is considered as the reference method (Deugnier et al., 1982). Biochemical method includes colorimetric and atomic absorption spectrophotometry (AAS), the latter being the reference method, which has many practical advantages and also has a different standard of reference (Deugnier et al., 1982).

Several scoring systems have been proposed. The Scheuer's scoring system, either in its original presentation (Scheuer et al., 1962) or modified according to Rowe et al. (1977) (Table 1) and Sindram and Marx (1988) (Table 2) is widely used because it is simple. However, it was not satisfactorily validated. The system proposed by the Deugnier and Turlin (2007) (Table 3) was well validated in both

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**Table 1**
Histological grading of iron storage from Searle et al. (2002).

| Grade | Ease of observation and magnification required |
|---|---|
| 0 | Granules absent or barely discernible at ×400 |
| 1 | Granules barely discernible at ×250 and easily confirmed at ×250 |
| 2 | Discrete granules resolved at ×100 |
| 3 | Discrete granules resolved at ×25 |
| 4 | Masses visible at ×10, or naked eye |

**Table 2**
Histological grading of iron storage from Sindram and Marx (1988).

| Grade | Localization of iron deposition in liver |
|---|---|
| 0 | No stainable iron |
| 1 | Iron in acinar zone 1 |
| 2 | Iron in acinar zones 1 and 2 |
| 3 | Iron in acinar zones 1, 2 and 3 |
| 4 | Iron in all acinar zones and in biliary epithelium |

hemochromatotic (Deugnier and Turlin, 2007; Imbert-Bismut et al., 1999) and non-hemochromatotic iron overload disorders (Ortega et al., 2005), but it remains mainly used for research purposes due to its relative complexity (Deugnier and Turlin, 2007). In few studies, histologic grading was compared with AAS or colorimetry (Li et al., 2002; Deugnier et al., 1982; Imbert-Bismut et al., 1999; Ortega et al., 2005; Turlin and Deugnier, 1998) for example in article (12) Deugnier's histological index is compared with the biochemical method ($r = 0.425$, $p < 0.004$).

The aim of our study is to introduce a new and rather simple scoring method for iron load in liver of thalassemic patients (MTK) taking into account both the granular size and zone of iron deposition which is similar but very much simpler than Deugnier's scoring system and to assess its validity by atomic absorption spectrophotometry. Further, Sindram and Marx scoring system was compared with our new method in this study.

**Table 3**
Histological grades of iron storage from Deugnier and Turlin (2007).

| | |
|---|---|
| Hepatocyte iron | 0, 3, 6, 9 or 12<br>According granules size<br>In each Rappaport area |
| Sinusoidal iron | 0, 1, 2, 3 or 4<br>According granules size<br>In each Rappaport area |
| Portal iron | 0, 1, 2, 3 or 4<br>According to % of iron overloaded macrophages, biliary cells, and vascular walls |
| Total iron score | 0–60 |

## 2. Materials and methods

Liver biopsies taken by percutaneous route in Markaze Tebbi Koodakan (children medical center affiliated with Tehran University of Medical Sciences) from year 2004 to 2009 were chosen. They were stained by hematoxylin-eosin, Perl stain for iron and trichrome stain (reticulin in some cases). All biopsies were assessed by one pathologist (F. Mahjoub). Histologic grading and staging was performed for each case according to modified HAI system. Iron scoring was performed according to Sindram & Marx (S&M) (Table 2) and MTK1–3 (Tables 4–6) scoring systems.

Paraffin blocks were processed for AAS (instrument model: GBC 932 AA, Australia), and the results of the iron analysis (HIC) were reported as μmol/g dry liver weight. Also, fresh tissue was submitted to the atomic energy (AE) laboratory at the time of biopsy. The iron content was determined by Proton-Induced X ray Emission (PIXE) and results were reported as times of normal value (example; 4.5 times of normal).

The statistical analysis was performed using SPSS, version 16 (SPSS Inc., Chicago, IL, USA). The Spearman rank order test was used to determine the nonparametric correlation between the histological study (grading, staging and iron scoring), biochemical findings

**Table 4**
Histological grades of iron storage—MTK1.

| Grade | Localization of iron deposition in liver | Grade | Iron granules density |
|---|---|---|---|
| 0 | No stainable iron | 0 | No visible iron Granule |
| 1 | Iron in acinar zone 1 | 1 | Fine and scattered Granules |
| 2 | Iron in acinar zones 1 and 2 | 2 | Larger Granules from 1 |
| 3 | Iron in acinar zones 1, 2 and 3 | 3 | Larger & dense Granules from 2 |
| 4 | Iron in all acinar zones and in biliary epithelium | 4 | Clumps of Granule |
| Final MTK scoring | | | |
| Coefficient score | | 4 | |
| Calculation method | | Zonal score × granular dencity score | |
| Minimum score | | 1 × 1 = 1 | |
| Maximum score | | 4 × 4 = 16 | |

**Table 5**
Histological grades of iron storage—MTK2.

| Grade | Localization of iron deposition in liver | Grade | Iron granules density | | |
|---|---|---|---|---|---|
| 0 | No stainable iron | 0 | No visible iron Granule | | |
| 1 | Iron in acinar zone 1 | 1 | Fine and scattered Granules | | |
| 2 | Iron in acinar zones 1 and 2 | 2 | Larger Granules from 1 | | |
| 3 | Iron in acinar zones 1, 2 and 3 | 3 | Larger & dense Granules from 2 | | |
| 4 | Iron in all acinar zones and in biliary epithelium | 4 | Clumps of Granule | | |
| Calculation method | | | | | |
| Zonal score | Granular density score | Final score | Zonal score | Granular density score | Final score |
| 1 | 1 | 1 | 3 | 1 | 9 |
| | 2 | 2 | | 2 | 10 |
| | 3 | 3 | | 3 | 11 |
| | 4 | 4 | | 4 | 12 |
| 2 | 1 | 5 | 4 | 1 | 13 |
| | 2 | 6 | | 2 | 14 |
| | 3 | 7 | | 3 | 15 |
| | 4 | 8 | | 4 | 16 |