Analysis of Iron Overload in Liver Biopsies

Aleena Jain, Manjusha Karegar, Amita Joshi, Rachana Chaturvedi, Chitresh Aggarwal

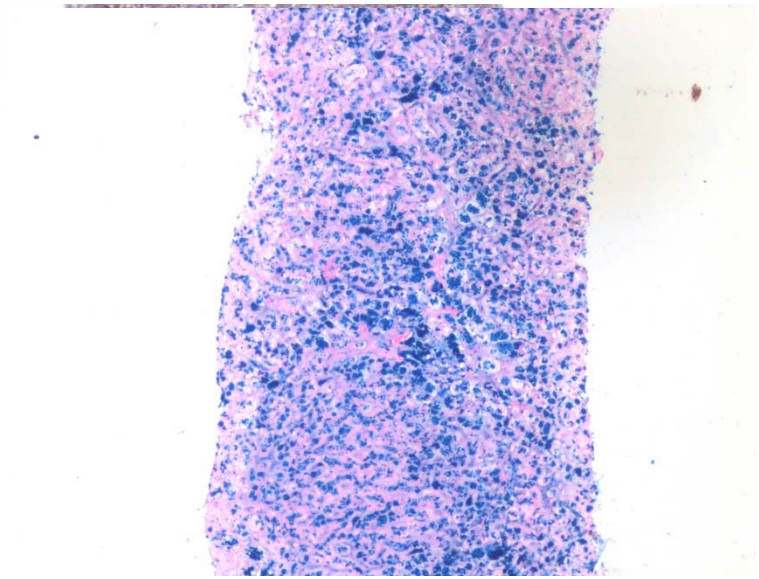
India

Introduction

- Liver disease is a major cause of morbidity & mortality worldwide
- Histological assessment of liver biopsy is cornerstone in the evaluation and management of patients with liver disease
- Integral component of the clinician's diagnostic armamentarium.
- Liver biopsy has three major roles; diagnosis, assessment of prognosis (disease staging) and to assist in making therapeutic management decisions.
- It plays an important role because the concepts and classifications of liver disease are rooted in morphology. While supplementary diagnostic modalities have been developed, none has replaced liver biopsy as the 'gold standard'. There has been a substantial increase in the understanding and interpretation of morphological changes across a whole range of liver diseases.

Stains

- H & E
- Masson's trichome
- Reticulin
- Prussian blue
- PAS, PAS-diastase
- Orcein



These should be re-classified as 'routine' & not 'special' as they are essential for liver biopsy interpretation

In this study, we aim to determine the prevalence of iron overload in the Indian population as demonstrated by Prussian blue stain on liver biopsy and its correlation with the morphological changes, with a special emphasis on fibrosis

Aims & Objectives

1.To determine the incidence and etiology of iron overload in liver biopsies

2. To evaluate the grade, parenchymal localisation and cellular distribution of hepatic iron overload

3. To correlate iron overload with the stage of fibrosis

Methods & Materials

- Retrospective analysis conducted in a tertiary care centre in western India
- All liver biopsies over a period of 30 months were evaluated and those biopsies with hepatic iron overload as evident by positive staining on Prussian blue were included in the study
- Diagnosis was determined from clinical and pathological correlation
- Clinical evaluation included history, physical examination and serological testing for known causes of liver disease
- Evaluation of the liver specimens for iron staining was performed in a blinded fashion

Study Design & Criteria

- **STUDY DESIGN:** Observational and cross sectional study
- **ELIGIBILITY CRITERIA:**

Inclusion criteria:

- Stainable iron evident on liver biopsy using Prussian blue method.
- Age more than 12 years

Exclusion criteria:

- Unequivocal positivity for Prussian blue
- Scanty tissue available for analysis
- Cases in which slides &/blocks couldn’t be reviewed

Study Sequence

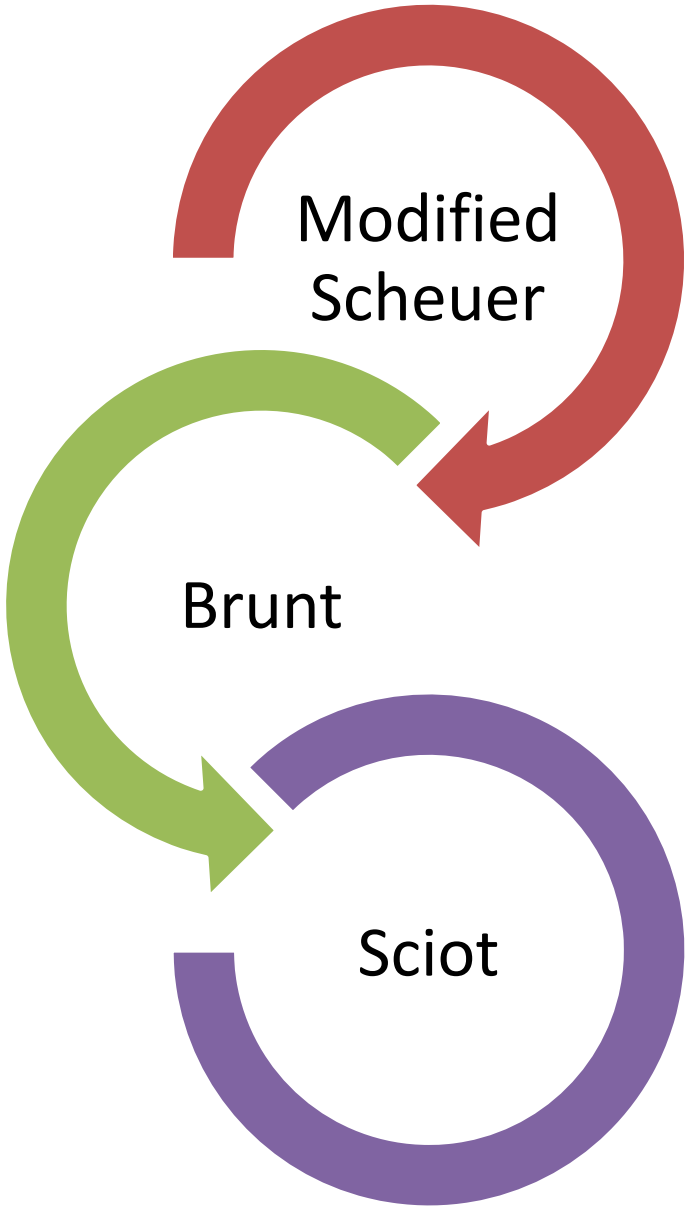
- The following patient parameters noted at the time of presentation were recovered
- Age , Sex, Registration number
- Clinical diagnosis,
- Significant radiological findings (X-Ray, USG, CT scan findings),
- Lab investigations, especially liver enzymes

The H&E, Masson Trichome, Reticulin, PB, PAS-D and orcein stained sections of the cases were retrieved & screened for conformation of diagnosis, grading and staging. All the special stains were reviewed with special emphasis on Prussian blue. Liver biopsies with positive Prussian blue staining were recruited for the study.

Analysis of iron deposited on Prussian blue stained sections

1. Pattern of distribution according to the localisation of iron deposition was assessed.
2. Cellular distribution of iron was studied.
3. Semi-quantitative grading of iron deposition, based on lens magnification for visualization of iron granules in hepatocytes, was performed

Grading of the hepatic iron overload

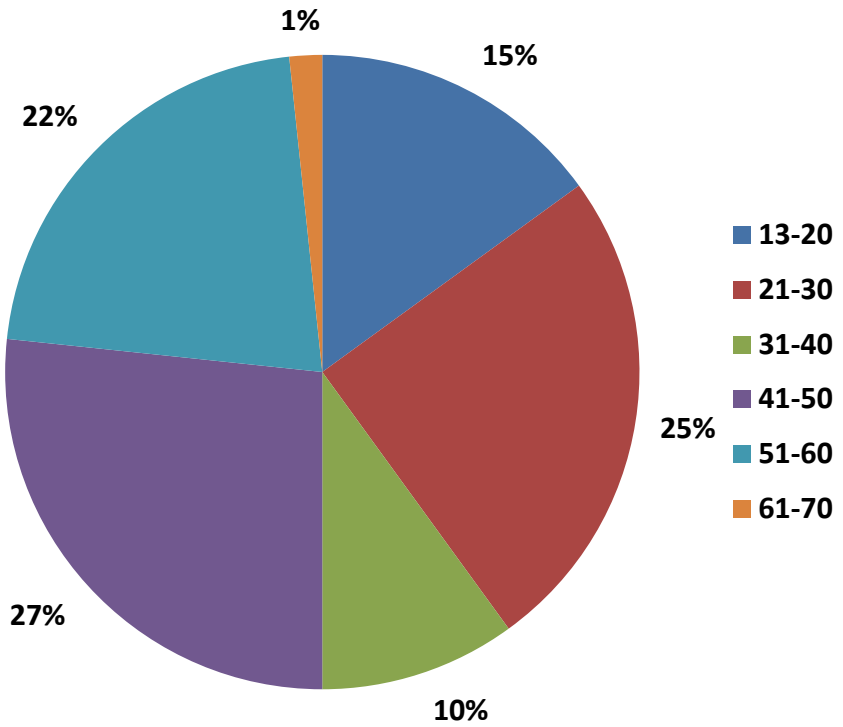


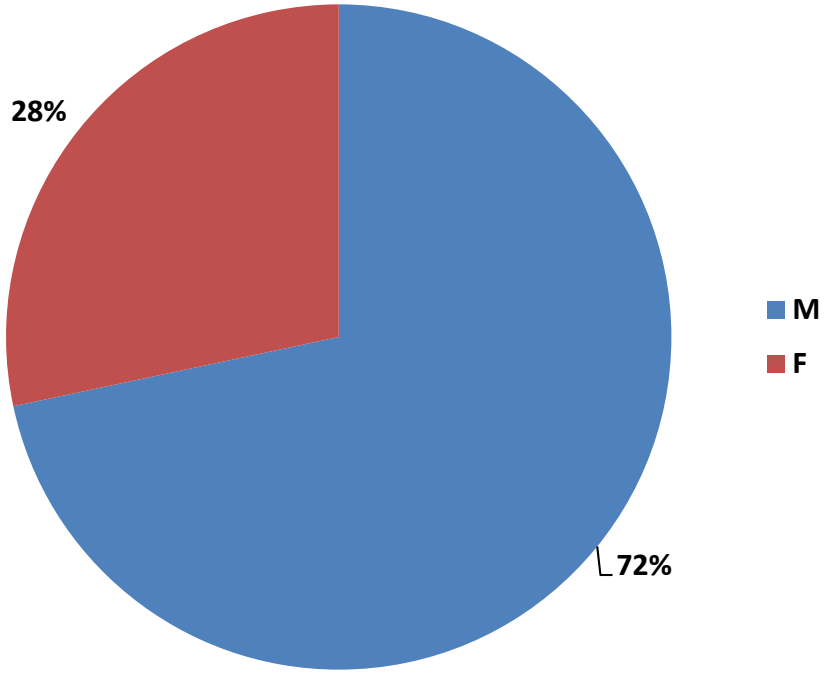
Gd	Scheuer	Brunt		Sciot		
		HH	Non-HH	HC	KC	PM
0	-/ barely visualized granules at x400			No iron	No iron	No iron
I	Few granules at x400	Any quantitative grade of Fe granule in scattered HC in acinar zone 1	Scattered sinusoidal lining cells / PM: + Occ zone 1 HC (faint granular) +	Fine focal granules	Fine focal granules	Fine focal granules
II	Numerous granules at x400	HC in >50% acinar zone 1 +; KC &/ PM ±	Panacinar KC/sinusoidal lining cell +; PM ±; Occ zone1 HC +	Fine granules (atleast) zonal	Fine granules in most KC	Scattered fine granules
III	Granules seen at x100	HC zone 1/ panacinar.Zonal gradient+. KC aggregates, PM + Biliary epi/vasc. endo +/-	Panacinar KC, PM, diffuse zone 1/ periseptal HC +	Coarse granules (zonal)	Coarse granules in hypertrophic KC	Coarsely clumped granules
IV	Granules at x40 /naked eye	Naked eye/ coalesced HC and KC granules (little /no gradient). Fe-free foci+; Biliary epi ,vascular endo+	As in 3, with iron deposition in fibrous tissue of portal tracts or septa	Diffuse coarse granules	Coarse granules in almost all KCs	Massive deposits

Results

Of the total 472 liver biopsies studied over a period of 30 months, 60 (12.7%) demonstrated hepatic iron overload on PB

Age & Gender Distribution

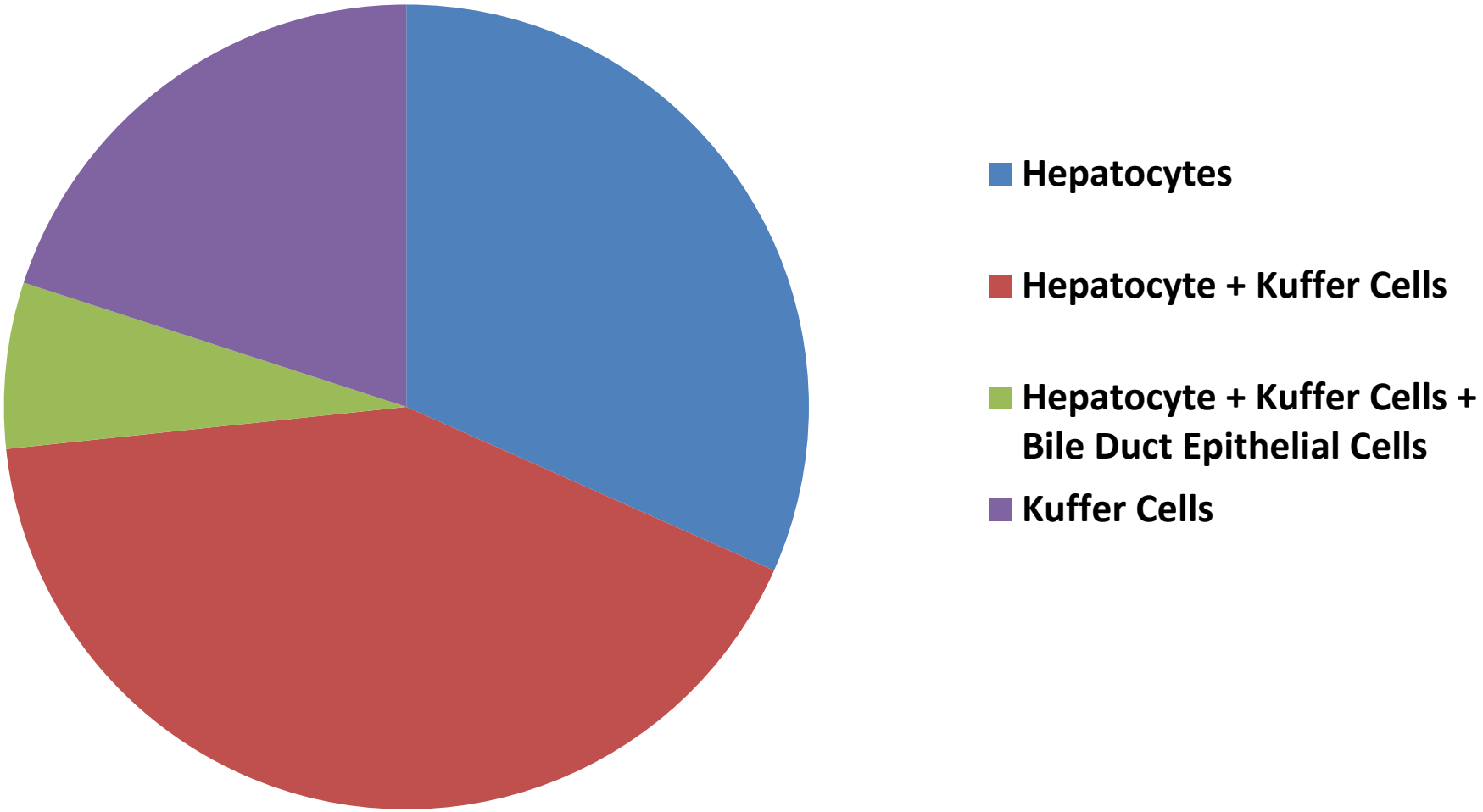




Hepatic Iron Overload: Distribution Pattern

Cellularity	No. of Cases	%age
Diffuse	38	63.3
Diffuse Hepatocytic only	8	13.3
Diffuse+Sinusoidal	14	23.3
Diffuse+Sinusoidal+Portal	4	6.7
Sinusoidal only	12	20
Zonal	14	23.3
Zone 1, 2 (with gradient)+Sinusoidal	3	5.0
Periportal (Zone 1)/ Periseptal	5	8.3
Periportal (Zone 1)/ Periseptal+Sinusoidal	6	10
Patchy	8	13.3
Total	60	100

Hepatic Iron Overload: Cellular Distribution



Comparison of Grade of Hepatic Iron Overload According to Different Methods

Criteria	Modified Scheuer		Brunt et al			Sciot et al	
Grade	No. of Cases	%	HH (n=38)	Non-HH (n=22)	%	No. of Cases	%
I	25	41.7	12	12	40	30	50
II	16	26.7	11	4	25	20	33.3
III	13	21.7	11	4	25	5	8.3
IV	6	10	4	2	10	5	8.3
Total	60	100	38	22	100	60	100

By Sciot's method, incidence of grade 1&2 hepatic iron was higher than grade 3&4

Correlation Of Grade of Iron Overload with Cellular Distribution

PB Grade	No. of Cases (n=60)			
	HC	KC	HC + KC	HC + KC+ Bile Duct Epi. Cells
1	7	10	8	-
2	8	1	7	-
3	4	1	8	-
4	-	-	2	4
Total	19	12	25	4

With increasing grade of iron deposition, there was involvement of multiple cell types.

Correlation Of Grade of Iron Overload with Pattern of Distribution

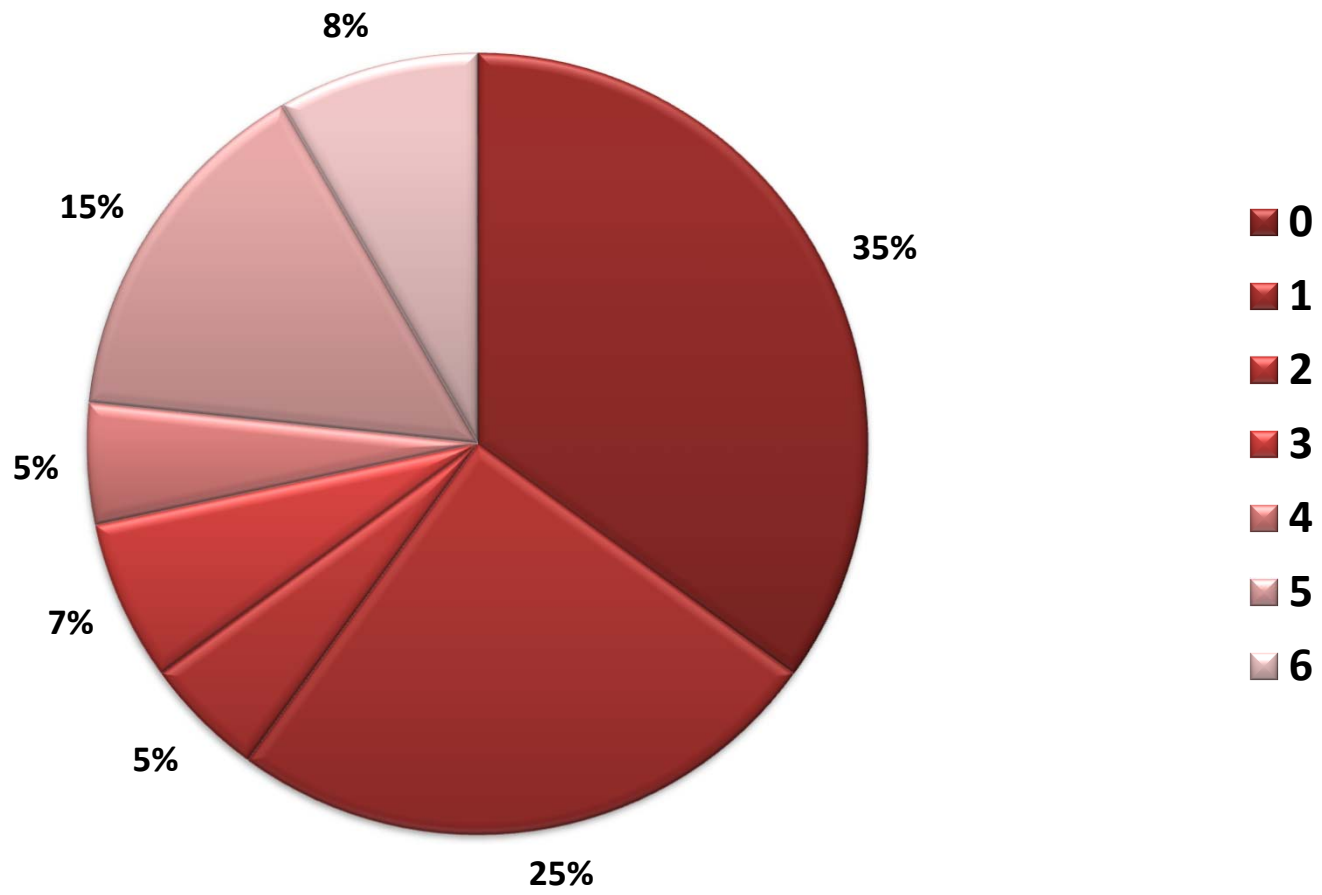
PB Grade	No. of Cases (n=60)				
	Diffuse (±Sinusoidal)	Zone 1,2 (±Sinusoidal)	Periportal (Zone 1)/ Periseptal (±Sinusoidal)	Sinusoidal only	Patchy
1 (n=25)	8	0	2	10	5
2 (n=16)	7	0	5	1	3
3 (n=13)	6	2	4	1	0
4 (n=6)	5	1	0	0	0
Total	26	3	11	12	8

The common pattern of distribution in grade 2, 3 and 4 was diffuse followed by periportal; whereas in grade 1 it was sinusoidal followed by diffuse.

Hepatic Iron Overload: Etiology

Diagnosis	Total No. of liver biopsies	No. of Prussian blue positive cases (%)
Acquired/ secondary iron overload		
Secondary Iron Overload (Thalassemia, Sickle cell anemia, Hereditary spherocytosis, Myelodysplastic syndrome)	08	08 (100%)
Iron overload in liver disease		
Hepatotropic viruses	133	17 (12.8%)
- HBV	124	13 (10.5%)
- HCV	3	1 (33.3%)
- HBV + HCV	3	1 (33.3%)
- HEV	3	2 (66.7%)
Non- Hepatotropic viruses	02	01 (50%)
NAFLD	34	03 (8.8%)
Steatosis/ Steatohepatitis (ASH / NASH)	09	02 (22.2%)
Submassive necrosis	04	01 (25%)
Chronic hepatitis (unknown etiology)	17	2 (11.7%)
Cryptogenic Cirrhosis	46	6 (13%)
Autoimmune liver disease	28	03 (10.7%)
Drug induced liver injury	17	04 (23.5%)
Miscellaneous		
NCPF	17	03 (17.6%)
Hepatic adenoma	01	01 (100%)
Normal liver histology		
Normal liver histology	61	9 (14.8%)
Others	95	-

Correlation of Hepatic Iron Overload with Stage of Fibrosis



Incidence of stainable hepatic iron in comparision with other studies

Authors	Zimmerman et al 1961	Barton et al 2015	Present study 2017
Incidence	227/558	22/83	60/472
%	40.7	26.5	12.7

There are very few studies in the literature which have given the overall incidence of hepatic iron overload. The incidence of hepatic iron overload in our study was lower than that of Zimmerman et al and Barton et al. which is possibly due geographical distribution as both these studies were from African population which African iron overload, formerly called Bantu siderosis, is an important pathology

Spectrum of aetiologies of hepatic iron overload in comparison with other studies

Diagnosis	Authors					
	Zimmerman et al 1961	Williams et al 1967	Martinelli et al 2003	Souza 2005	Nelson 2011	Present study 2017
Acquired /Secondary Iron Overload	2/2 (100%)	-	-	-	-	8/8 (100%)
Viral Hepatitis	35/66 (53%)	-	-	-	-	17/133 (12.8%)
- HCV	-	-	-	30/95 (31.5%)	-	1/3 (33.3%)
- HBV	-	-	19/39 (48.7%)	-	-	13/124 (10.5%)
-Non- Hepatotropic viruses	1/5 (20%)	-	-	-	-	1/2 (50%)
NAFLD	-	-	-	-	293/849 (34.5%)	3/34 (8.8%)
Steatosis/ Steatohepatitis	21/66 (31.8)	-	-	-	-	2/9 (22.2%)
Cryptogenic Cirrhosis	36/98 (36.7%)	6/11 (55%)	-	-	-	6/46 (13%)
Normal liver histology	30/122 (26%)	-	-	-	-	9/61 (14.8%)

- On comparing the grade of iron overload with the stage of fibrosis, we did not find any correlation.
- Though various authors have described increased incidence of fibrosis with hepatic iron overload, the correlation of histological grade with increasing stage of fibrosis cannot be made, possibly due to various confounding factors.
- In the study by Martinelli et al, the predominant pattern was HH 9/19 (47.3%), followed by non–HH in 6 (31.5%) and mixed in 4 (21.2%). This was similar to our study in which amongst HBV +ve cases, 8/14 (57.1%) showed HH, while the rest showed non-HH pattern

Limitations

This is a retrospective work in which the biochemical tests for iron studies and DNA analyses to detect mutation of iron-associated genes could not be done which has limited the clinico-pathological correlation.

Summary & Conclusion

- This was a retrospective study of 60 liver biopsies with hepatic iron overload as evident by positive staining on PB(out of total 472 liver biopsies) over a period of 30 months.
- The age group of patients with hepatic iron overload ranged from 12 to 62 years, with a mean age of 37.9 years.
- Male preponderance was observed with the male to female ratio being 2.5:1.
- According to the modified Scheuer criteria, Grade 1 prussian blue staining was most commonly observed (41.7%) followed by grade 2 (28.3%). grade 4 was the least common (10%).
- The most common pattern of hepatic iron distribution observed was diffuse (63.3%) followed by zonal (23.3%) and patchy (13.3%).
- Cellular iron distribution was commonest in the HC (80%) either exclusively or along with other cells (KC ± bile duct epithelial cells) followed by deposition only in the KC (20%).
- All cases with acquired/ secondary Fe overload showed high grade Fe deposition (grade 3&4) while most cases with underlying liver pathology showed low grade Fe deposition (grade 1& 2).

- With increasing grade of iron deposition, there was involvement of multiple cell types.
- The common pattern of distribution in grade 2, 3 and 4 was diffuse followed by zonal; whereas in grade 1 it was sinusoidal followed by diffuse.
- The hepatic iron grading by Scheuer's method was comparable with Brunt's method, while it was not with Sciot's method.
- The most common underlying liver pathology in iron overload was hepatotropic virus infection (n=17), followed by cryptogenic cirrhosis (n=6) and drug induced liver injury (n=4). Normal liver histology was seen in 9 cases and acquired/ secondary iron overload in 8 cases.
- Majority of patients with hepatic iron overload showed evidence of fibrosis (65%), the commonest being stage 1.
- There was no significant correlation with the grade of hepatic iron overload and the stage of fibrosis.
- The detection of Fe deposition in liver biopsies is important and its presence implies the need for closer follow-up of the patient.

Thank You