Original Article

Hematoxylin and eosin stain shows a high sensitivity but sub-optimal specificity in demonstrating iron pigment in liver biopsies

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Abstract

Background: Perls' stain is routinely used to demonstrate iron in liver biopsies. We tested the hypothesis that it may be unnecessary in cases, where no iron or another similar pigment was seen on the routine hematoxylin and eosin (H and E) stained section. **Aim:** The aim of this study was to evaluate the efficiency of H and E stain in demonstrating iron in liver biopsies as well as to determine the possibility of replacing Perls' stain with H and E stain. **Materials and Methods:** Two hundred pairs of slides of liver biopsies were taken from the archival files of the Department of Pathology from 2006 to 2011. Perls' and H and E slides were independently reviewed for the presence of iron. **Results:** Hundred and one cases showed the presence of iron using H and E stain. 84 of 86 cases showed positive iron using both Perls' and H and E stains. Seventeen cases were positive using H and E stain but negative with Perls'. Only two cases did not show the presence of iron using H and E stain. Ninety-seven cases were negative using both Perls' and H and E stains. H and E stain showed a sensitivity, specificity, accuracy, positive predictive valve, and negative predictive value of 97.67%, 85.08%, 90.5%, 83.16%, and 97.98%, respectively. **Conclusion:** We demonstrate that the H and E stain is a sensitive method to detect iron pigment in liver biopsies, particularly when present in large quantities. A negative H and E stain might obviate the need for extra Perls' staining, thus saving costs and shortening report turn-around times.

Key words: Hematoxylin and eosin stain, iron, liver biopsy, Perls' stain

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Introduction

Liver biopsy presents a very useful tool in the diagnosis and management of various liver diseases. Most histological laboratories use Perls' stain as a routine special stain to evaluate the amount of iron present in liver biopsies. In Oman, the examination of liver biopsies is also a routine histopathological test counting for about 102 biopsies a year.

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Hematoxylin and eosin (H and E) stain, which is the most widely used histological stain, gives an excellent general morphological picture of the nucleus, and cytoplasmic details. Iron can be seen in H and E stain as a gold - brown granules in macrophages.[1] However, most histopathologists prefer Perls' or Prussian blue stain to evaluate the presence of iron in liver biopsies as considered to be the gold standard. In Perls' stain, iron is released by acid hydrolysis using hydrochloric acid. Then, potassium ferrocyanide detects iron and produces dense blue precipitates. The forming precipitate is insoluble in acid and, therefore, acid solutions are used as counterstains.[2] The aim of this study was to evaluate the efficiency of H and E stain in demonstrating iron in liver biopsies as well as to determine the possibility of replacing Perls' stain by H and E stain.

Materials and Methods

This study was ethically approved by the Medical Research Committee and Ethics Committee (#519). Slides of Perls' and H and E stains of liver biopsies were taken from the archival files of the Department of Pathology from 2006 to 2011.

Totally, 238 pairs of slides (Perls' and H and E) were found and 38 pairs were excluded because they had insufficient materials and so 200 pairs were obtained. Briefly, in all pairs, liver biopsies were first fixed in 10% neutral buffered formalin for 24 h and histoprocessed. The blocks were then cut into sections of 3 μm thickness using a rotary microtome. The sections were stained with Harris H and E and Perls' stains.[2] For each batch of Perls' stains, a known positive control was treated as with the test.

All the slides were reviewed independently by three investigators. In Perls' stain, the positive result of iron was defined by detecting blue deposits as an intracellular pigment. While in H and E stain, the iron deposits were defined by detecting golden - brown pigments as intracellular granules. Formalin pigment was excluded from the assessment as it stains deep brown to black color.

The degree of staining of iron in Perls' and H and E stains was graded by the following criteria;[3] absent, trace, sparse, moderate, and abundant.

Results

Of the 200 cases, 97 were negative using both Perls' and H and E stains while 84 were positive by both stains. The remainder had discordant results: Seventeen were positive by H and E but negative with Perls' (false positives). H and E stain missed iron in 2 cases, which were positive on Perls' [Table 1]. Of these 17 cases, 8 were graded as trace, 8 as sparse, and one was graded as moderate [Table 2]. H and E stain revealed 97.67% sensitivity, 85.08% specificity, 90.5% accuracy, positive predictive valve 83.16%, and 97.98%, negative predictive value [Table 3]. 18 out of 33 (54%) cases were graded as a trace in both Perls' stain and H and E stain [Figures 1 and 2]. Furthermore, 21 cases (100%) showed an abundant iron with both stains [Figures 3 and 4].

Discussion

The findings of this study showed that H and E stain has 97.7% sensitivity and 85.08% specificity in demonstrating iron pigment in the examined liver biopsies. These data are in line with our recent study showing that H and E stain was sensitive in 86% in demonstrating iron pigment in bone marrow trephine biopsies.[4] Another study showed a sensitivity of 70% for the detection of iron pigment in bone marrow trephine biopsies using H and E stain.[5] It is noteworthy that the studies on liver biopsy to evaluate iron pigment on Perls' and H and E stained slides are very scanty. However, very few studies were performed using trephine bone marrow biopsies.

Hematoxylin and eosin staining demonstrates a good morphological details of nucleus, and cytoplasm. On the other hand, Perls' stain is only used to demonstrate iron pigment. Nuclear and cytoplasmic detail are lacking with Perls' stain. In addition, Perls' stain requires to be prepared fresh, consumes time, and the reagents are costly. If one slide can combine all the histopathological details including the demonstration of iron, it will subsequently save histologist and pathologist time.

Only 2% showed the absence of iron using H and E stain while Perls' stain showed positive reaction for iron pigment. There are many reasons for the absence of iron in the liver biopsies using H and E stain; difficulties to visualize small pigments, inability to show all content of iron, and probably the color of H and E stain overrides the iron brown color. In fact, this finding is in line with other similar studies which showed higher percentage in which Perls' staining of the bone marrow trephine biopsies was positive, but no iron was seen on the H and E stained sections.[4,5] Despite the Perls' stain representing an extra step to be performed, it is preferred due to its simplicity and specificity in detecting blue iron pigment in a red background and excluding mimics.

Table 1: Comparison of H and E and Perls' staining methods on the liver biopsy sections

	Perls' stain (positive)	Perls' stain (negative)	Total
H and E stain (positive)	84	17	101
H and E stain (negative)	2	97	99
Total	86	114	200

H and E: Hematoxylin and eosin

Table 2: Comparison of the grade of iron between H and E and Perls' staining methods

	H and E stain					Total
	Absent	Trace	Sparse	Moderate	Abundant	
Perls'stain						
Absent	97 (98%)	8	8	1	0	114
Trace	2	18 (54%)	0	1	0	21
Sparse	0	6	15 (62.5%)	1	0	22
Moderate	0	1	1	19 (82.6%)	0	21
Abundant	0	0	0	1	21 (100%)	22
Total	99	33	24	23	21	200

H and E: Hematoxylin and eosin

Table 3: Accuracy of H and E staining method in the diagnosis of 200 liver biopsies

Parameter	Value (%)
True positive	84 (42)
True negative	97 (48.5)
False positive	17 (8.5)
False negative	2 (1)
Sensitivity	97.67
Specificity	85.08
Accuracy	90.5
Positive predictive value	83.16
Negative predictive value	97.98

H and E: Hematoxylin and eosin

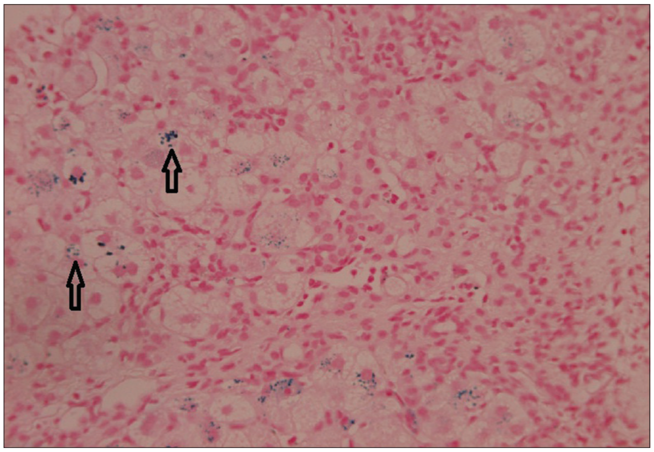

Figure 1: Perls' stain showing iron pigment in liver biopsy (trace grade) (×40)

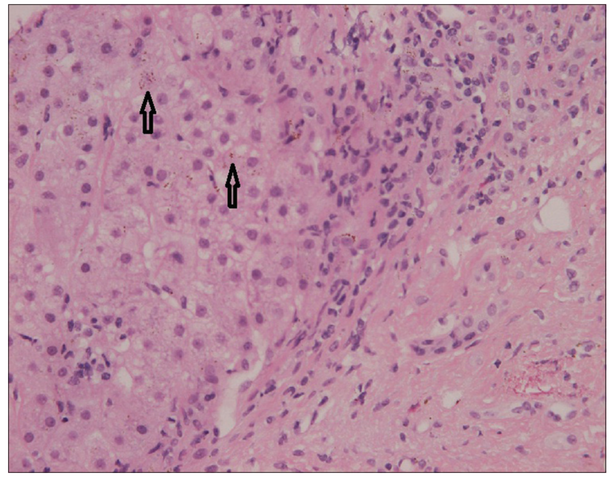

Figure 2: H and E stain showing liver pigment in liver biopsy (trace grade) (×40)

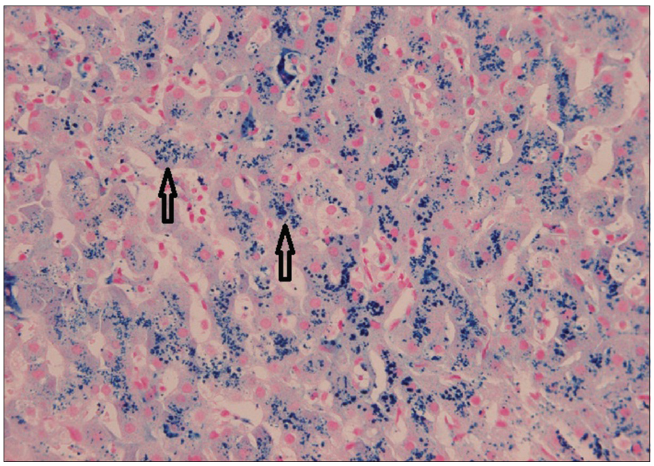

Figure 3: Perls' stain showing iron pigment in liver biopsy (abundant grade) (×40)

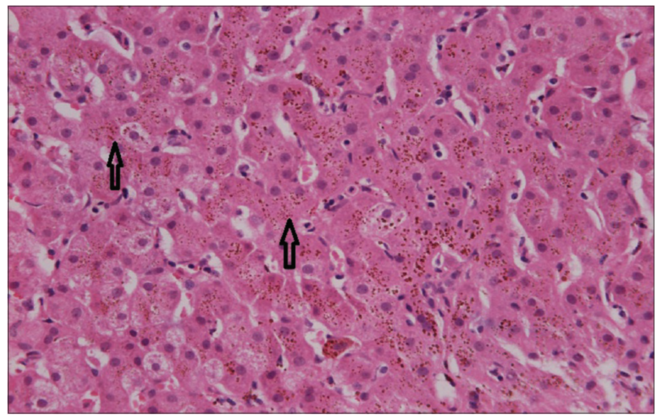

Figure 4: H and E stain showing iron pigment in liver biopsy (abundant grade) (×40)

Fixation is an important factor in the evaluation of iron content in the liver biopsies. In this study, 10% neutral buffered formalin for 24 h was used as a standard fixative. Thus, the formation of formalin pigment was unlikely to occur. If it occurs, it is easy to identify formalin pigment as mentioned previously.

The 17 cases which showed only brown pigments in Perls' stain and were thought to contain iron in H and E stain would demonstrate as other pigments like lipofuscin or bile pigments which also have a golden brown color but are not iron. These pigments can lead to mis-diagnosis as iron pigment in H and E stain and can be distinguished from iron by their location.

Conclusion

We demonstrate that the H and E stain is a sensitive method to detect iron pigment in liver biopsies, particularly when present in large quantities. A negative H and E stain might obviate the need for extra Perls' staining, thus saving costs and shortening report turn-around times.

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