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Capability of Hematoxylin and Eosin Stain to Demonstrate Hemosiderin in Bone Marrow Trephine Biopsy

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Abstract

Background: Perl's stain is routinely used to demonstrate hemosiderin in bone marrow trephine biopsies. However, it is time consuming and costly. The purpose of this study was to evaluate the efficiency of Hematoxylin and Eosin (H&E) stain in demonstrating hemosiderin in bone marrow trephine biopsies as well as to determine the possibility of replacing Perl's stain by H&E stain.

Methods: One hundred and eleven pairs of slides of bone marrow trephine biopsies were taken from the archival files of the Department of Pathology of Sultan Qaboos University Hospital, Sultanate of Oman, from 2008 to 2009. Perl's and H&E slides were independently reviewed for the presence of hemosiderin.

Results: 71 cases showed the presence of hemosiderin using Perl's stain. 61 of 71 cases showed positive hemosiderin using H&E stain. Only 10 cases showed negative hemosiderin using H&E stain. The remaining 40 cases were negative using both Perl's and H&E stains.

Conclusion: The findings of this study showed that H&E stain is efficient and sensitive enough to evaluate hemosiderin in bone marrow trephine biopsies when present in large quantities but not in small quantities.

Keywords: Hemosiderin; Perl's stain; Bone marrow trephine biopsies; Hematoxylin and eosin stain; Decalcification

Introduction

Most histological laboratories use Perl's stain as a routine special stain to evaluate the amount of iron present in bone marrow trephine biopsies. In Oman, the examination of bone marrow trephine biopsies is also a routine histopathological test counting for about 208 biopsies a year. In fact, iron deficiency is considered to be a major disease affecting many people worldwide [1]. Although the examination of bone marrow trephine biopsies is invasive, uncomfortable and expensive, it is considered to be the gold standard for evaluating iron status in patients suffering from bone marrow diseases [2].

The measurement of serum ferritin, serum iron, total iron binding capacity, mean corpuscular volume and mean corpuscular hemoglobin for the evaluation of iron stores is less reliable [1,3]. However, the evaluation of bone marrow aspiration, which is usually performed in parallel with bone marrow trephine biopsies, gives more hematological details that are more beneficial for histopathologists [2]. It is known that free iron is toxic and it may lead to the formation of free radicals. Thus, iron is stored in two forms: ferritin or hemosiderin. Hemosiderin, which is found in macrophages and degraded hemoglobin, is mainly associated with iron overload [4].

Hematoxylin and Eosin (H&E) stain, which is the most widely used histological stain, gives an excellent general morphological picture of nucleus and cytoplasmic details. Hemosiderin can be seen in H&E stain as a gold – brown granules in macrophages [5]. However, most histopathologists prefer Perl's or Prussian blue stain to evaluate the presence of hemosiderin in bone marrow trephine biopsies. In Perl's stain, hemosiderin is released by acid hydrolysis using hydrochloric acid. Then, potassium ferrocyanide detects hemosiderin and produces dense blue precipitates. The forming precipitate is insoluble in acid and therefore acid solutions are used as counterstains [4]. Perl's stain is a time consuming and costly. Thus, the aim of this study was to evaluate the efficiency of H&E stain in demonstrating hemosiderin in bone marrow trephine biopsies as well as to determine the possibility of replacing Perl's stain by H&E stain.

Methods

This study was ethically approved by the Medical Research Committee and Ethics Committee (MREC # 408) from the College of Medicine and Health Sciences, Sultan Qaboos University, Sultanate of Oman. Slides of Perl's and H&E stains of bone marrow trephine biopsies were taken from the archival files of the Department of Pathology of Sultan Qaboos University Hospital from 2008 to 2009. 127 pairs of slides (Perl's and H&E) were found and 16 pairs were excluded because they had insufficient materials and so 111 pairs were obtained. Briefly, in all pairs, bone marrow trephine biopsies were first fixed in 10% neutral buffered formalin for 24 hours. Biopsies were then decalcified overnight in a Gooding and Stewart fluid [4]. The blocks were then cut into sections of 3 μ m thickness using a rotary microtome. The sections were stained with Mayer's H&E and Perl's stains [4]. For each batch of Perl's stains, a known positive control was treated as with the test.

All the slides were reviewed independently by two investigators. In Perl's stain, positive result of hemosiderin was defined by detecting blue

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deposits either as intracellular or extracellular pigment. Blue artifact pigments, not hemosiderin, were distinguished by their locations on the top of the bone marrow biopsy sections. While in H&E stain, the hemosiderin 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 colour.

The degree of staining of hemosiderin in Perl's and H&E stains was graded by the following criteria [6]; 0: absent, 1: trace, 2: sparse, 3: moderate, 4: abundant and abnormal

Results

Table 1 shows that there were 61 cases out of 71 when both stains (Perl's and H&E) show positive results. Also, there were only 10 cases shows negative results with H&E stain while they were positive in Perl's stain. Moreover, the table shows there were 40 cases out of 50 when H&E stain gave negative results same as Perl's stain. Furthermore, the possibility of errors during Perl's staining is absent as there was no case showed positive result with H&E stain but negative with Perl's stain.

Table 2 shows that 33.8% of bone marrow trephine biopsies had equal grade of hemosiderin. However, 63.4% of the cases showed that Perl's stain was superior to H&E stain, especially in grade 1 to 3 (Figures 1 and 2). Surprisingly, 2 cases (2.8%), mainly in grade 4 (Figures 3 and 4), showed that H&E stain was superior to Perl's stain in demonstrating hemosiderin.

	Perl's stain (positive)	Perl's stain (negative)	Total
H&E stain (positive)	61	0	61
H&E stain (negative)	10	40	50
Total	71	40	111

Table 1: Comparison of H&E stain and Perl's stain on bone marrow biopsy sections.

Grade	No. of slides that had same grade in Perl and H&E	No. of slides when Perl was superior to H&E	No. of slides when H&E was superior to Perl
1	12	23	0
2	3	8	0
3	3	8	0
4	6	6	2

Table 2: Number of slides when Perl's stain grade was equal, superior or inferior to grade of H&E stain.







Figure 2: H&E stain showing hemosiderin pigment in bone marrow biopsy (grade 1) (x40)



Figure 3: Perl's stain showing hemosiderin pigment in bone marrow biopsy (grade 4) (x40).



Figure 4: H&E stain showing hemosiderin pigment in bone marrow biopsy (grade 4)(x40).

Discussion

This study showed that H&E staining is sensitive (86%) in demonstrating hemosiderin in the examined bone marrow trephine biopsies. The findings of this study slightly disagreed with previous study which showed that only 70% (71 out of 101 cases) sensitivity of H&E staining for the detection of hemosiderin in bone marrow trephine biopsies [7]. H&E staining, which is a routine stain that used for every specimen including bone marrow trephine biopsies, demonstrates good morphological details of nucleus and cytoplasm. On the other hand, Perl's stain is only used to demonstrate hemosiderin pigment. Nuclear and cytoplasmic detail are lacking with Perl's stain. In addition, Perl's 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 hemosiderin, it will subsequently save histologist and pathologist time.

Perl's stain is more specific than H&E stain for the detection of hemosiderin in bone marrow trephine biopsies. 14% showed negative hemosiderin in H&E stain while Perl's stain showed all positive for hemosiderin. This finding is in line with other similar study, which showed that 29.7% in which Perl's staining of the bone marrow trephine biopsies was positive but no hemosiderin was seen on the H&E stained sections [7]. Despite the fact that Perl's stain is costly and time consuming, it is preferred by histopathologists due to its simplicity in detecting blue hemosiderin in a red background using a low magnification.

It is worth mentioning that H&E stain did not detect hemosiderin when it is present in small quantity in the bone marrow trephine biopsies. There are many reasons for the absence of hemosiderin in the bone marrow trephine biopsies using H&E stain; difficulties to visualize small pigments, inability to show all content of hemosiderin, confusion between hemosiderin and formalin pigments, and probably the colour of H&E stain over rides the hemosiderin brown colour.

Throughout this study, it was taken into consideration, the presence of other artifacts that might be present in Perl's and H&E stains. Blue artifact pigments, not hemosiderin, were distinguished by their locations. There are many reasons which could lead to the formation of these artifacts such as the mixture solutions of Perl's stain that was left for prolonged time after mixing and before filtration, no filtration used, insufficient washing of sections after staining or probably not using fresh solutions. Regarding H&E staining, formalin pigment was distinguished from hemosiderin by staining deep brown to black colour and randomly distributed.

Fixation and decalcification are important factors in the evaluation of hemosiderin content in the bone marrow trephine biopsies. In this study, 10% neutral buffered formalin for 24 hours 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. Recent study was carried out to evaluate the effects of three decalcifying agents (30% formic acid with formaldehyde and NaCl, 33% formic acid with formaldehyde and water and 5% nitric acid) on liver and lung tissues for 24, 48, 72 and 96 hours [8]. The findings showed that hemosiderin content in liver and lung tissues was significantly reduced using nitric acid while both agents of formic acid showed reduction in hemosiderin but statically insignificant. The current study did not evaluate the effect of formic acid on the bone marrow trephine biopsies as this decalcifying agent is routinely used and shows satisfactory results for H&E, special stains and immunohistochemical markers. In addition, formic acid is considered to be a weak acid, slow in action and causes less damage [9]. In fact, the effect of decalcification on hemosiderin staining is controversial. Some authors found that hemosiderin to be better stained with Perl's stain in bone marrow trephine biopsies [10]. On the contrary, others found hemosiderin to be reduced or even absent using Perl's stain [5,11].

As a limitation of this study, we should point out the absence of positive controls of bone marrow trephine biopsies that had a known amount of hemosiderin prior to decalcification. This would indicate the positive or negative effects of formic acid on Perl's and H&E stains. In conclusion, the findings of this study showed that H&E stain is efficient and sensitive enough to evaluate hemosiderin in bone marrow trephine biopsies when present in large quantities but not in small quantities.

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