Original Article

Comparison of Hematoxylin-eosin Staining and Methyl Violet Staining for Displaying Ghost Cells

Wenxin Zhang, Yongping Li, Jianxian Lin, Shangtao Wan, Huanhuan Gao, Lanzhi Zhang, Jianliang Zheng, Ping Zhang*

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, China

Abstract

Purpose: To compare the merits and limitations of hematoxylin-eosin (HE) and methyl violet staining for displaying ghost cells from vitreous or aqueous humor.

Methods: A specimen containing ghost cells was adjusted to five different concentrations: (12×10^4, 10×10^4, 8×10^4, 6×10^4 and 4×10^4 cells/ml) and subjected to smearing and methyl violet and HE staining. The staining results were observed by light microscopy.

Results: The ghost cells were readily observed at a cell density of > 8×10^4 cells/ml with methyl violet staining, but only a few cells were occasionally seen at lower cell densities. In contrast, ghost cells were seen at all cell densities with HE staining.

Conclusion: Methyl violet staining is more rapid and simpler for the identification of ghost cells, but its staining color more readily fades, the slides cannot be stored, and it is only effective at a cell density of > 8×10^4 cells/ml. In contrast, HE staining is more time-consuming but it can display cell morphology and distinguish cell components more explicitly and slides can be permanently stored. HE staining has advantages over methyl violet staining in detecting the ghost cells when the concentration is < 8×10^4 cells/ml.(***Eye Science 2013; 28: 140-143***)

Keywords: ghost cells; cell counting; methyl violet staining; hematoxylin-eosin staining

A ghost cell is an enlarged rigid degenerating red blood cell. Compared with normal red blood cells, it loses the biconcare configuration and flexibility to become a spherical shape with a hollow center. Ghost cells fail to pass through the trabecular meshwork of the anterior chamber angle, which causes obstruction and secondary ghost cell glaucoma. Patients mainly present with the following clinical manifestations: new or old vitreous hemorrhage, anterior chamber hemorrhage, sharp IOP elevation after several days to weeks, no neovascularization in the iris, many red or yellowish brown minute particles floating within aqueous humor, yellowish white pseudohypopyon, open chamber angle, yellowish brown deposits seen in trabecular meshwork tissues, and KP(-)[1-3]. In the present study, aqueous or vitreous humor was collected for the detection of ghost cells to confirm the diagnosis.

Materials and methods

The samples containing ghost cells were diluted to different concentrations, smeared, and stained with methyl violet or HE staining for viewing with a light microscope.

Cell count

1.The prepared solutions were diluted to 12×10^4, 10×10^4, 8×10^4, 6×10^4 and 4×10^4 cells/ml. 2.A blood count plate (0.10 mm, 1/400 mm^2) was covered with a 20× 20 mm glass cover slip and a droplet of the test solution was added along the lower edges of the bilateral grooves of the glass slide so that it gradually infiltrated into the counting region. 3.Cell counting was performed using an inversion microscope (10 ×). The ghost cells in 4×16 square chambers were counted three times and the values were averaged. The cell density was calculated from the formula: cell count/ml=4 square chambers/4×10^4.

Methyl violet staining

1.The syringe containing the sample was kept vertical and still for 10 to 20 minutes with the tip down-

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* **Corresponding author**: Ping Zhang, E-mail: pings@163.com

ward.2.The glass slides were cleaned, disinfected with 75% ethanol, and then dried. 3.A mixture containing the cell solution and 1% methyl violet at a ratio of 5:1 was dropped onto the glass slide, evenly mixed, and then gently covered with a glass cover slip. 4. The sample was observed with a light microscope.

HE staining

The 1st and 2nd steps were the same as described for methyl violet staining. 3.One droplet of test solution was added to the glass slide and dried at room temperature.4.The sample was then fixed in methanol solution for 30 minutes. 5.The methanol solution was removed, and the sample was irrigated with distilled water and subjected to conventional HE staining.

Results

Methyl violet staining: ghost cells were bluish violet and shaped like empty spheres, slightly larger than normal red cells, and the cell membranes were mildly thickened. Unevenly stained dark blue particles were observed within and among red cells and on the cell membrane. The spherical cells were observed slightly floating in the light microscope field. Small amounts of cellular debris and methyl violet deposits were also seen.

HE staining: the background of the glass slide was clean and clear, the cell walls of ghost cells appeared red, revealing a round shape. Membrane rupture and defects were noted in partial cells. Red cellular debris was observed unevenly within the cytoplasm and red particles were dispersed among the cells. Other cell components, such as lymphocytes and neutrophilic leukocytes, were also seen.

Staining at different cell concentrations: ghost cells stained with methyl violet were clearly shown at cell densities of 12×10^4 cells/ml and 10×10^4 cells/ml (Figure 1); ghost cells were occasionally seen at a cell density of 8×10^4 cells/ml (Figure 2) and no ghost cells were observed at cells densities of 6×10^4 cells/ml and 4×10^4 cells/ml (Figure 3). Ghost cells stained with HE were detected at all five cell densities tested (Figures 4–6).

Discussion

In humans, mature red blood cells are flexible and oval biconcave disks with a diameter of approximately 7 μm. The cell membrane is flexible, so red blood cells can pass through gaps of 3-5 μm in diameter. Therefore, in the anterior chamber, they can traverse the trabecular meshwork gap and enter the canal of Schlemm. When red blood cells degenerate into ghost cells, they lose all hemoglobin except for

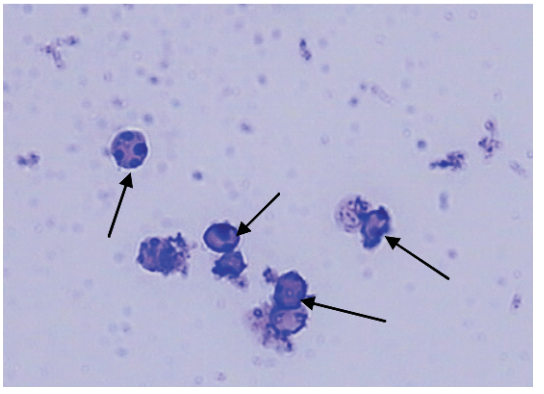

Figure 1 The arrows show ghost cells stained with methyl violet at a cell density of 12×10^4 (×400)

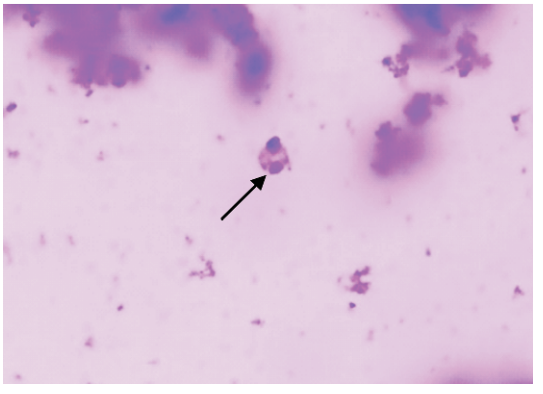

Figure 2 The arrow shows ghost cells stained with methyl violet at a cell density of 8×10^4 (× 400)

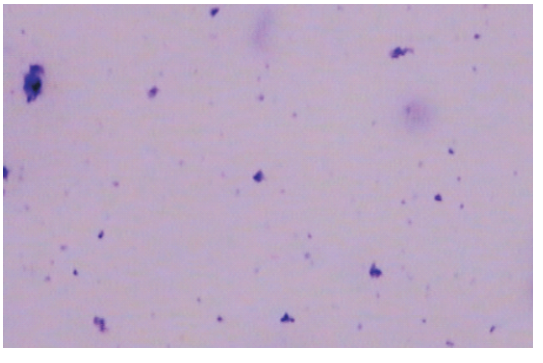

Figure 3 No ghost cells were seen following methyl violet staining at a cell density of 4×10^4 (×400)

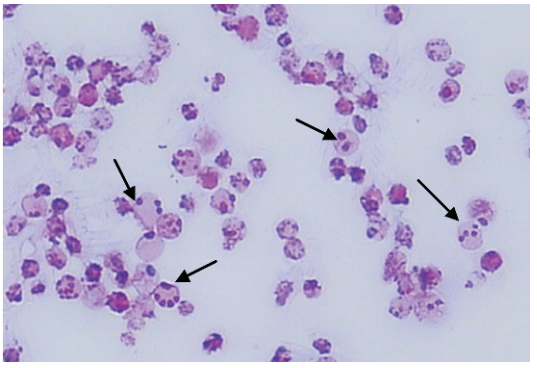

Figure 4 The arrows show ghost cells stained with HE at a cell density of 12×10^4 (×400)

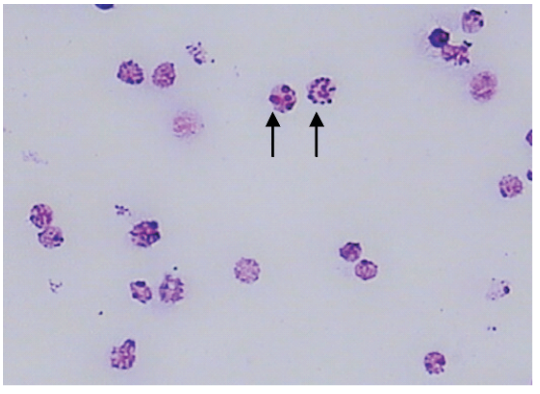

Figure 5 The arrows show ghost cells stained with methyl violet at a cell density of 8×10^4 (×400)

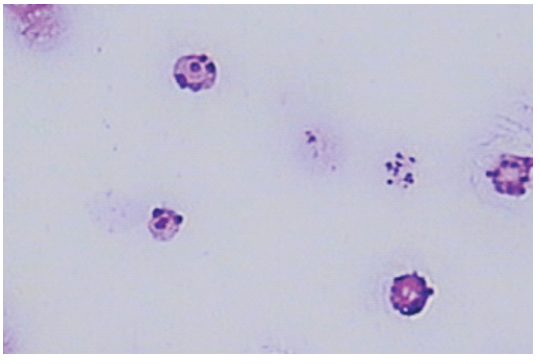

Figure 6 The arrows show ghost cells stained with HE at a cell density of 4×10^4 (×400)

a slight amount on the periphery or in the middle of cell body. The cells swell and lose their biconcave confignration, becoming rigid spherical form. These changes mean that ghost cells fail to traverse the trabecular meshwork of the anterior chamber angle, thereby leading to obstruction and even secondary ghost cell glaucoma. Ghost cells have the following characteristics: 1. They originate from a variety of intraocular hemorrhages, especially traumatic vitreous hemorrhage; 2. They are able to traverse the defects of vitreous anterior membrane into the anterior chamber; 3. Ghost cells entering the anterior chamber can mechanically obstruct the trabecular meshwork, leading to POAG with acute IOP elevation[4,5].

Patients with ghost cell glaucoma yielded aqueous humor or vitreous samples of approximately 0.1 ml, which were clear, yellow and had no deposits. The syringe was maintained vertical and still for 10 to 20 minutes to allow the degenerated red blood cells to form a globin sediment. The sample was then applied as a droplet onto the edge of a glass slide, from where it gradually infiltrated into the count region. The liquid covered all count regions and no bubbles were seen. The liquid quantity, approximately 5 μl, did not overflow the edge of the glass slide. The globin in ghost cells had a relatively strong affinity with 1% methyl violet solution. The ghost cells were stained blue and the cell shape and structure could be clearly seen with the light microscope. Methyl violet staining is quick and simple for the identification of ghost cells but the staining color fades and the glass slides cannot be stored. A slight amount of stained impurities may be observed, making it difficult to distinguish the ghost cells. Previous studies revealed that the optimal ratio of sample solution and methyl violet stain was 5∶1[6,7], which was subsequently confirmed by our findings in this study. Identification of the ghost cells at a low cell density was difficult, which may cause misdiagnosis or missed diagnosis. Cell counting revealed the difficulty in distinguishing ghost cells at a cell density< 8×10^4 cells/ml whereas a mild amount of dye sediment could be seen. The number of ghost cells visible by light microscopy significantly increased when the cell density exceeded 8×10^4 cells/ml. Ghost cells could be readily observed following HE staining at a wide range of cell densities from 4 to 12 cells/ml. Other cell types, such as lymphocytes, neutrophilic leukocytes, and epithelioid cells, were also seen due to the lucid background of HE staining. HE staining was more time-consuming, but it is applicable over a wide range of cell densities, especially for trace quantities of ghost cells. HE staining can also identi-

fy cell components of other cells and the glass slides can be permanently preserved.

Disclosure statement

There is no conflict of interest to declare.

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