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Differentiation of mulberry bodies (cells) from oval fat bodies using distinct fat staining methods

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ABSTRACT

Objective: The detection of mulberry bodies (cells) in urine facilitates the early diagnosis of Fabry disease. However, their detection is difficult because of their very small size and various shapes when stained with Sternheimer's staining. We investigated methods to specifically detect the various appearances of mulberry bodies (cells).

Methods: The urinary sediments of patients with Fabry disease were subjected to Sternheimer's staining, and the mulberry bodies (cells) and oval fat bodies were compared using four fat staining methods (Sudan III, Oil Red O, Nile blue, and Sudan black B).

Results: Differentiation between mulberry bodies (cells) and oval fat bodies using Sternheimer's, Sudan III staining was difficult. In contrast, they were clearly identified and distinguished when Nile blue and Sudan black B staining was performed. From the perspective of differentiating from OFBs, Sudan III and Oil Red O seemed more useful, as they show clear differences in stainability. On the other hand, for positively staining mulberry cells themselves, Nile blue and Sudan black B appear useful.

Conclusions: Mulberry bodies (cells) have various appearances. Although they are difficult to distinguish from oval fat bodies, they can be identified using Sudan III and Oil Red O, Nile blue and Sudan black B staining.

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Key Words
mulberry body, Fabry disease, urinary sediment, fat staining

INTRODUCTION

Fabry disease is a rare lysosomal storage disorder caused by a deficiency of the lysosomal enzyme alpha-galactosidase A (α-galactosidase A; α-GAL), resulting in the accumulation of glycolipids such as globotriaosylceramide (GL-3) in various cells [1)2)]. Accumulation of glycolipids in the urine of these patients results in the formation of circular, berry-like structures called mulberry bodies (cells). The detection of mulberry bodies in urine has been used as an indicator for early diagnosis of this disease [3)4)]. When stained with the Sternheimer method, mulberry bodies (cells) in urinary sediments exhibit a whorl-like appearance, making it an inexpensive and sensitive method used in routine laboratory practice [5)]. However, it is difficult to detect mulberry bodies (cells) and differentiate them from oval fat bodies (OFBs) using this method because they are very small and have various appearances.

In the present study, we evaluated staining methods to improve detection and differentiation of mulberry bodies (cells).

MATERIALS AND METHODS

Clinical Specimens

Urine specimens from three patients with Fabry disease, admitted to Tokai University Hospital, were analyzed in this study. The diagnosis of Fabry disease was confirmed by demonstration of deficiency in α-GAL activity. Oval fat bodies were obtained from urine samples of patients with nephrotic syndrome, specifically those with diabetic nephropathy

This study was approved by the Institutional Review Board for Clinical Research of Tokai University Hospital (14R-022) and conformed to the principles outlined in the Declaration of Helsinki.

Appearance of Mulberry Bodies (Cells) After Sternheimer Staining

The Sternheimer staining method was applied to urinary sediments and examined at 400 × magnification, as routinely used in our laboratory. The microscopic findings of mulberry bodies (cells) and urinary sediments were compared.

Comparison of Four Fat Staining Methods

Four fat staining methods were used to compare the stainability: Sudan III, Oil Red O, Nile blue, and Sudan black B (Muto Pure Chemicals Co., Ltd. Tokyo, Japan). Sudan black B staining was performed by mixing the stock solution with preservation buffer at a ratio of 3:2, and the solution was filtered prior to use. For staining, 10 mL of the patient's urine was centrifuged for 5 min at 500 × g, the supernatant was removed using an aspirator, and several drops of the stain solution were added to the sediment. The staining times for each staining method were as follows: Sudan III at 37 ℃ for 60 minutes; Oil Red O at 37℃ for 15 minutes; Nile blue at 60℃ for 20 minutes, followed by staining at room temperature for 20 minutes; and Sudan black B staining at 37℃ for 30 minutes. After staining, a drop of the sediment was placed on a glass slide. A coverslip was used, and stainability was observed under a microscope. Staining of mulberry bodies (cells) was compared with that of OFBs.

RESULTS

Appearance of Mulberry Bodies (Cells) After Sternheimer Staining

The results of Sternheimer staining are shown in **Figure 1**. The mulberry bodies (cells) exhibited various appearances (**Figures 2A** and **2B**). Differentiating mulberry bodies (cells) from fat granules and OFBs using Sternheimer's staining was difficult because they were small and closely resembled each other. The stainability of urine in these three patients did not differ.

Comparison of Four Fat Staining Methods

The results of fat staining, including Sudan III, Oil Red O, Nile blue, and Sudan black B, are shown in **Figure 3**. The colors of the OFBs obtained using each staining

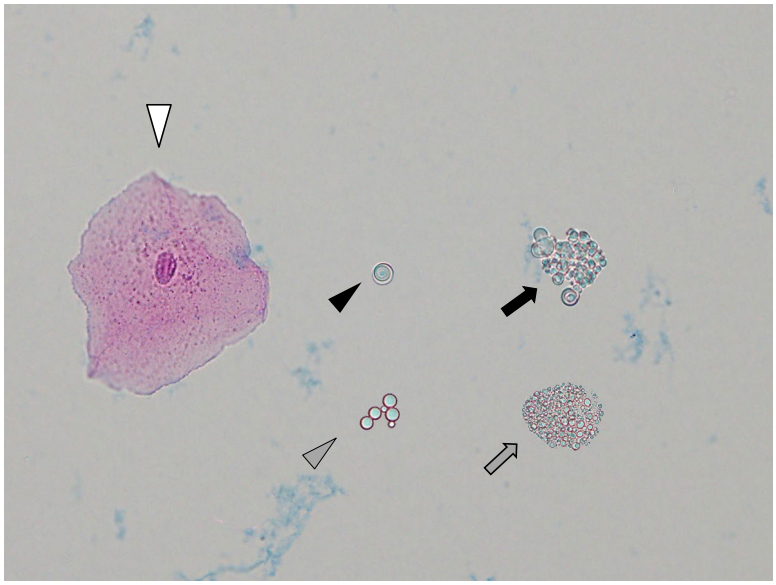

Figure 1 Comparison of mulberry bodies (cells) and other urinary sediment constituents using Sternheimer's staining (high-power field, 400×).
Mulberry bodies (black arrowhead), mulberry cells (black arrow), fatty granules (gray arrowhead), oval fat bodies (gray arrow), and squamous cells (white arrowhead).

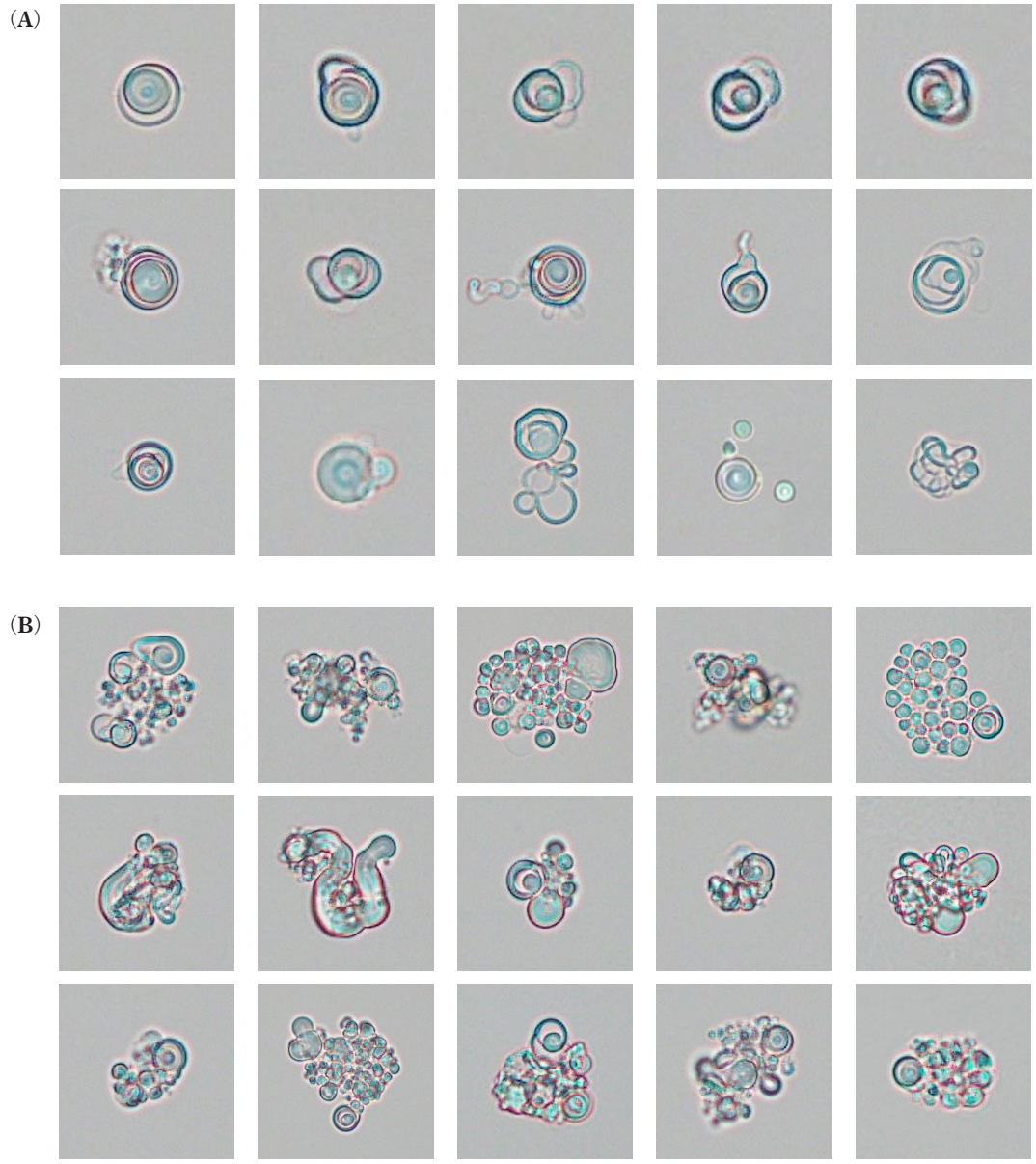

Figure 2 Appearance of mulberry bodies (cells) after Sternheimer's staining.
Mulberry bodies (**A**) and cells (**B**) showed different appearances. They resemble fatty granules and oval fat bodies, making differentiation difficult.

method were orange, brown, intense blue, and blue. Furthermore, the mulberry bodies (cells) appeared pale lemon yellow, pale red or unstained, or blue or black. The identification of mulberry bodies (cells) using Sudan III and Oil Red O staining was difficult; however, they were easily identifiable using Nile blue and Sudan black B staining. The staining results were consistent across the three patients.

As shown in **Figure 3** (**A**), differentiation between mulberry bodies (cells) and OFBs was challenging when using Sudan black B, as both exhibited a similar blue coloration. In contrast, Sudan III and Oil Red O staining produced more pronounced color differences between the two structures, aiding their distinction in routine microscopy (high-power fields). Furthermore, **Figure 3** (**B**) summarizes that Sudan III, Oil Red O, Nile blue and Sudan black B stainings were shown. Nile blue, Sudan black B yielded intense coloration of the mulberry bodies (cells), which facilitated their positive identification (**Table 1**).

DISCUSSION

Mulberry bodies (cells) are characteristically whorl-like in appearance [3)–6)]. However, their appearance varied, and staining mulberry bodies with Sudan III and Oil Red O was difficult. Sudan III and Oil Red O staining

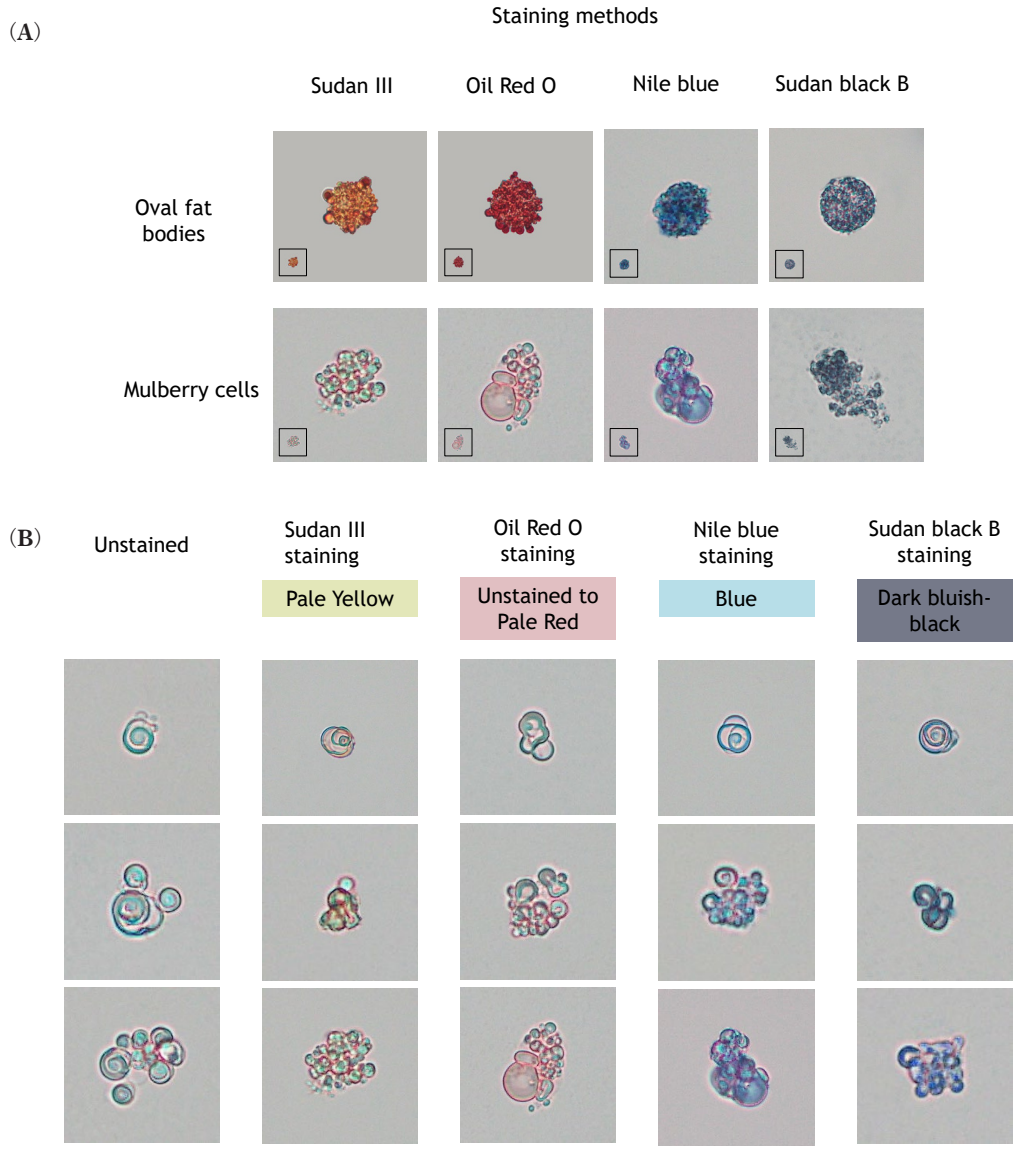

Figure 3 Comparison between oval fat bodies and mulberry cells.
The results of four types of fat staining are shown: Sudan III, Oil Red O, Nile blue, and Sudan black B. The colors of the oval fat bodies after fat staining were orange, brown, strongly blue, and blue (**A**). Mulberry bodies are approximately 8μm in size and extremely small. The lower left of Figure 3(**A**) shows mulberry cells under high-magnification microscopy. The mulberry cells were pale lemon-yellow, pale red, blue, and dark bluish-black. The oval fat bodies were stained orange to red with Sudan III and Oil Red O, whereas the mulberry bodies (cells) were weakly stained (**B**).

Table 1 Staining affinity of lipid-containing structures: mulberry bodies versus oval fat bodies.

Staining method	Structure	Staining characteristics	Lipid composition
Sudan III / Oil Red O	Mulberry bodies	Weakly stained or unstained	Globotriaosylceramide (GL-3)
	Oval fat bodies	Strongly stained	Triglycerides, Cholesterol esters
Nile Blue / Sudan black B	Mulberry bodies	Strongly stained	GL-3
	Oval fat bodies	Strongly stained	Triglycerides, Cholesterol esters

methods, which are often used for detecting OFBs in patients with nephrotic syndrome. As OFBs are composed of triglycerides and cholesterol esters, they are well stained with Sudan III and Oil Red O staining methods. In contrast, Nile blue and Sudan black B staining were more suitable for detecting mulberry bodies (cells) because they are composed of glycolipids. However, as illustrated in **Figure 3**(**A**), Sudan black B may not al-

ways be optimal for differentiating mulberry bodies from OFBs because both can show similar staining patterns. In such cases, Sudan III and Oil Red O, as demonstrated in **Figure 3**(**A**), offer clearer contrast for differentiation. Also, Nile blue as summarized in **Figure 3**(**A**)(**B**), was valuable for confirming the presence of mulberry bodies because of the intense and distinctive coloration of their glycolipid-rich structures. The colors of the oval fat bodies after fat staining were orange, brown, strongly blue, and blue (A). Mulberry bodies are approximately 8 μm in size and extremely small. The lower left of **Figure 3**(**A**) shows mulberry cells under high-magnification microscopy. The mulberry cells were pale-yellow, pale red, blue, and dark bluish-black. The oval fat bodies were stained orange to red with Sudan III and Oil Red O, whereas the mulberry bodies (cells) were weakly stained (B). Nile blue or Sudan black B staining following common fat staining (Sudan III or Oil Red O) facilitated the detection of mulberry bodies (cells). These morphological examination points are useful for improving the detection of mulberry bodies (cells) in laboratory practice. **Figures 3**(**A**) and (**B**) serve as visual guides summarizing the relative advantages of each staining method, offering a practical reference for laboratory technicians when encountering ambiguous lipid-containing structures in urinary sediments. Incorporating such a guide into routine diagnostic workflows may help reduce misidentification and improve the early detection of Fabry disease.

Conflict of Interest Statements

All authors declare that they have no conflicts of interest.

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