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ORIGINAL ARTICLE

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Evaluation of a new fluorescent reagent, fluorescent brightener 85, for the diagnosis of suspected onychomycosis compared with potassium hydroxide

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Summary

Onychomycosis is a common fungal infection in dermatology clinics. The commonly used diagnostic method, potassium hydroxide (KOH) direct microscopy, does not have a high-positive detection rate. Therefore, a new diagnostic method is needed to confirm onychomycosis. The objective of this research was to use fluorescent brightener 85 (FB 85), a new fluorescent reagent for fungi detection, and to evaluate this new method based on the diagnostic positive rate, accuracy and efficiency in suspected onychomycosis compared with the KOH method. Subungual debris was collected from clinically suspected onychomycosis cases and divided into two parts. The parts were examined by FB 85 and 10% KOH solution, respectively. In total, 108 patients with suspected onychomycosis were recruited. The positive rates of FB 85 and KOH were 88.9% and 55.6%, respectively. The patients showed significant statistical differences ($P < .001$). Compared with the KOH method, the FB 85 fluorescent method was an accurate and efficient method with a higher positive rate. This method could increase the diagnostic accuracy and efficiency of onychomycosis detection.

KEYWORDS
10% KOH method, direct microscopy, fluorescent brightener 85, fluorescent method, onychomycosis

1 | INTRODUCTION

Onychomycosis, caused by the dermatophyte, *Candida* or non-dermatophyte mould, is a common nail disease, and 10% KOH direct microscopy is the commonly used method for diagnosis, but it does not have a high-diagnostic positive detection rate.[1-3] Several reports have shown a relatively higher diagnostic positive rate using PAS (periodic acid-Schiff), but it has seldom been used in clinical practice due to its time-consuming nature compared with the KOH method.[1-3] The fluorescent method using CFW (calcofluor white) is a simple, rapid, highly efficient direct microscopy method that has a higher positive detection rate compared with KOH in various superficial fungal infectious diseases.[4,5] Several studies have compared CFW and other methods to diagnose suspected onychomycosis, but they did not show much better results with CFW.[6,7] Besides, the biggest disadvantages of CFW are that it diminishes quickly and its relatively bright background, but no alternative fluorescent reagents have been developed and used in the diagnosis of onychomycosis. Although some fluorescent reagents, such as Filipin, FM4-64, Solophenyl Flavine 7GFE 500 and Pontamine Fast Scarlet 4B[8-10], have been used to observe cultured fungi, they have never been applied in clinical practice. Therefore, finding and testing a new fluorescent reagent as an alternative or to supplement CFW to confirm onychomycosis is necessary. The authors are the first to evaluate a new fluorescent reagent, FB 85 (fluorescent

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brightener 85), a popular and widely used fluorescent brightener in wood, washing and dyeing industry in the world, in the detection of fungi in superficial fungal infectious diseases and proved that it had a higher positive rate than the KOH method with statistically significant differences (data not published). Therefore, in this study, we continued to evaluate the positive rate, accuracy and efficiency of FB 85 in the diagnosis of onychomycosis compared with KOH.

2 | MATERIALS AND METHODS

2.1 | Methods

Patients with suspected onychomycosis were recruited from the department of Dermatology in Beijing Tian Tan Hospital, Capital Medical University from October 2016 to May 2017. The surgical knife was burned on alcohol lamp first, and then the surgical knife and nails were disinfected with 75% alcohol. After disinfected, the debris samples were taken from infected nails immediately and divided into two equal parts on two slides. One slide was added a drop of 10% KOH, another was added with ª drop of FB 85 and KOH mixed solution. Next, the slides were heated and examined by two different technicians in two different rooms, respectively. The results and images were record and photographed, respectively, too.

2.2 | Reagents and equipment

In this experiment, 10% KOH and 0.1% FB 85 solutions were made by the authors. The FB 85 solutions (Shandong Yousuo Chemical Technology Co., Ltd., Shandong, China) were prepared as 0.2% (w/v) stock solutions in distilled water, with 10 mL of 0.2% FB 85 solution combined with 10 mL of 10% KOH. The KOH solution was prepared as a 10% (w/v) stock solution in distilled water. Microscopic observations were conducted with an Olympus CX23 LEDRFSIC microscope using either the 340 nm-360 nm ultraviolet light mode or the visible light mode.

2.3 | Data analysis and statistical analysis

The result was considered positive when the sample contained round or filamentous forms of fungal hyphae or spores with bright blue peripheral fluorescence with FB 85 method. The positive diagnosis with KOH method was the presence of fungal hyphae, arthroconidia, pseudohyphae, and spores with high refractivity. Then the positive rates of the FB 85 fluorescent method and the KOH method were calculated and compared. The statistical analysis was performed with the McNemar test using SPSS 21 (IBM SPSS Statistics, USA).

3 | RESULTS

One hundred eight specimens were obtained from one hundred eight patients with suspected onychomycosis, including 50 females and 68 males, and 82 affected toenails and 36 fingernails. Among the 108 specimens, 88.9% (96 specimens) were positive by the FB 85 fluorescent method, and 55.6% (60 specimens) were positive by the KOH method. A total of 55.6% (60 specimens) of the specimens was positive by both methods, and 11.1% (12 specimens) were negative by both methods. A total of 33.3% (36 specimens) of the specimens was positive by FB 85 but negative by KOH. In contrast, none of the specimens was negative by FB 85 but positive by KOH. These results are summarised in Table 1 and Figure 1.

The images from fluorescence microscope were similar to those with CFW. The fungal colour with FB 85 after excitation was blue or blue-green, which was easy to distinguish from the background keratinocytes, even when the keratinocytes were not dissolved well. The fungal structures remained clear, especially compared with the KOH method. Moreover, the fungal structure was still clear with a blurry or crystalline background after 3 hours when the stained slide was placed in the room environment (data not shown). Besides, the fluorescence was still strong while capture image or direct illuminate for several minutes. The images from different optical sources with the same microscope in the same position using the FB 85 method are compared in Figure 2. The hyphae and spores under ultraviolet light were clearer than under visible light.

4 | DISCUSSION

In our research, we analysed the diagnostic positive rates in 108 suspected onychomycosis cases using the FB 85 and KOH methods, and the positive rates were 88.9% and 55.6%, respectively. This result

TABLE 1 Positive and negative results of the two methods

	KOH positive	KOH negative	Total number
FB 85 positive	60	36	96
FB 85 negative	0	12	12
Total number	60	48	108

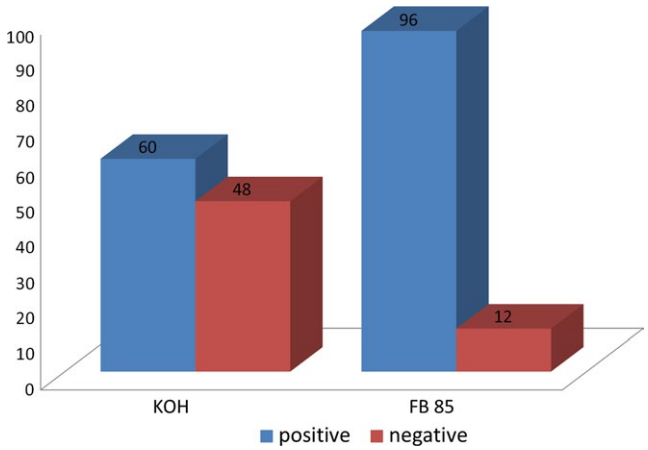

FIGURE 1 Positive and negative results of the KOH method and the FB 85 method in the diagnosis of 108 suspected onychomycosis cases

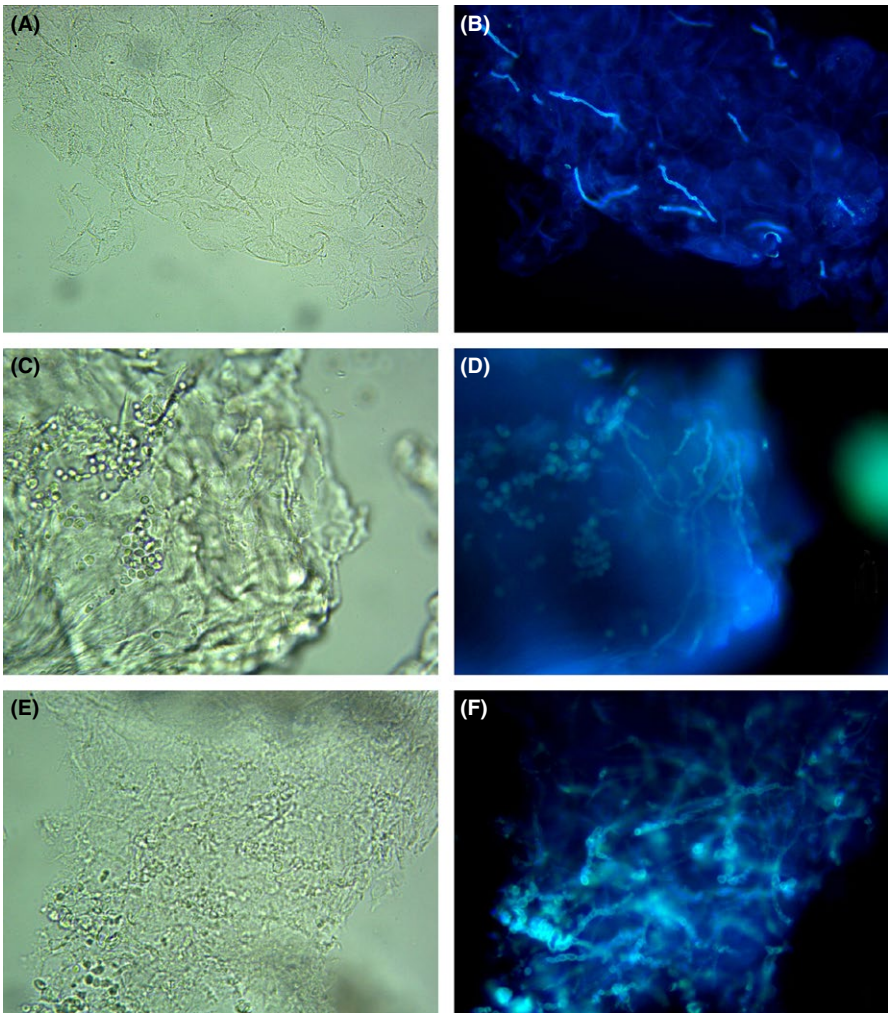

FIGURE 2 Microscopic images of the same field under visible light (A, C, E) and ultraviolet light (B, D, F) with the FB 85 method in an onychomycosis sample. The hyphae and spores under ultraviolet light were clearer and more obvious than under visible light. (× 400)

demonstrated that using the FB 85 method, a new fluorescent reagent, was better than using the KOH method and could increase the diagnostic positive rate for onychomycosis. This result has never been reported before. Compared with the KOH method, the FB 85 method not only had a higher diagnostic positive rate but also shortened the preparation time and observation time. Due to the close combination of FB 85 and chitin in the fungal cell wall and the weak connection between FB 85 and the background keratinocytes, the contrast between the fungi and the background remained obvious, even when the dissolution of keratinocytes was very poor or after several hours. Moreover, the fluorescence faded more slowly when it was illuminated for several minutes. Consequently, the FB 85 fluorescent method was faster and more efficient than the traditional KOH method, which could be helpful given the extensive clinical workload requirements.

Potassium hydroxide (KOH) is the most commonly used method for the diagnosis of onychomycosis. Because it is difficult to collect subungual debris, when the dissolution was poor, the positive rate of the KOH method was not satisfactory in previous reports with results of 41.5% (47 specimens positive among 108 specimens),[1] 56.4% (79 specimens positive in 140 specimens),[3] and 55.9% (52 specimens positive in 93 specimens).[2] These results of KOH were similar to our study, which had a 55.6% positive rate for the KOH method. The FB 85 method had an 88.9% positive rate. Therefore, the FB 85 method showed a higher diagnostic positive rate, leading to more accurate treatment.

In several previous reports that compared fluorescent methods using CFW and KOH for the diagnosis of onychomycosis, CFW did not have a much better result. Bonifaz et al[6] summarised 33 suspected onychomycosis cases, and the positive rates of CFW and KOH were 57.88% and 66.67%, respectively, which showed a lower result using CFW method. Weinberg et al[7] evaluated 105 suspected onychomycosis cases, and the CFW method showed a 72% positive rate, and the KOH method showed a 60% positive rate, which showed that CFW method was only a little higher than KOH method. In our study, the positive rate of the FB 85 method was much higher than KOH with a significant statistical difference. Moreover, FB 85 was much stable and faded slowly compared with CFW. Considering the identical mechanisms of CFW and FB 85, CFW should theoretically show similar results to FB 85 and a better result than KOH. These differences between studies may be related to fewer specimens or the technician's professional skill level. Therefore, in the future, we should compare KOH, CFW and FB 85 together to get more information.

The price of FB 85 should also be considered in clinical practice. No commercial FB 85 reagent is available in market up to now, but it

is much easy and economical to get the FB 85 because it is a widely used fluorescent brightener in wood, washing and dyeing industry in the world and is also very cheap. Therefore, FB 85 can be easily gotten and made by the laboratory. Moreover, KOH solution is often made by the laboratory. Therefore, compared with KOH method, FB 85 method does not increase the financial cost.

In conclusion, compared with the KOH method, the use of FB 85, a new fluorescent reagent for the detection of fungi, could increase the recognition rate of fungi and shorten the preparation time and the diagnostic time in suspected onychomycosis. This property is the largest advantage of the FB 85 method. Moreover, FB 85 is more stable than CFW. Therefore, FB 85 is a good, efficient fluorescent reagent as an alternative or to supplement CFW in the detection of fungi. The FB 85 fluorescent method was a simpler, faster and more convenient method for the diagnosis of onychomycosis and could increase the diagnostic accuracy and efficiency.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethical Committee for Human Study, Beijing Tian Tan Hospital, Capital Medical University. All the subjects who participated in the study signed an informed consent.

AUTHORS' CONTRIBUTION

All authors have read and approved the final manuscript. XY participated in the experimental designing, collaboration and drafting of the manuscript. Xiuling Y gave the idea of using FB 85 and supplied the reagent and the fluorescence microscope. AW participated in modifying the manuscript and the experimental design. QL supported for recruiting the patients. WH processed the KOH and FB 85 examination.

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