รายงานผู้ป่วย

PAS stained corneal scrapings in fungal keratitis.

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We compared PAS stain on corneal scrapings with conventional KOH stain in the diagnosis of fungal keratitis. Sixteen cases were included in the study; nine of them proved to be cases of fungal keratitis. PAS stain yielded 88.89% sensitivity compared with 44.44% by KOH stain. We conclude that PAS stain is superior to KOH stain in the diagnosis of fungal keratitis.

Key words : *Fungal keratitis, PAS stain, KOH stain, GMS stain, Gram stain.*

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ศึกษาผลของการวินิจฉัยโรคติดเชื้อของกระจกตาดำจากเชื้อรา โดยการใช้สีย้อมพีรีโอดิค แอซิด ชิฟ เปรียบเทียบกับโปตัสเซียมไฮดรอกไซด์ จากผู้ป่วย 16 คน ที่ทำการศึกษา พบว่าผู้ป่วย 9 คน เป็นโรคติดเชื้อกระจกตาดำจากเชื้อรา โดยสีย้อมพีรีโอดิค แอซิด ชิฟ สามารถวินิจฉัยได้ 8 คน คิดเป็น 88.89% แต่โปตัสเซียมไฮดรอกไซด์ สามารถวินัยฉัยได้ 4 คน คิดเป็น 44.44% ผลสรุปจากการวิจัยครั้งนี้ สรุปได้ว่าสีย้อมพีรีโอดิค แอซิด ชิฟ ดีกว่า โปตัสเซียมไฮดรอกไซด์ในการวินิจฉัยโรคติดเชื้อของกระจกตาดำจากเชื้อรา

One of the most common causes of keratitis in Thailand is fungal infection. Morbidity of fungal keratitis is greater than that of bacterial keratitis because the diagnosis is sometimes delayed. Management is difficult and restricted by the availability of effective antifungal agents and the extent to which they can penetrate into the corneal tissue.[1-3] Most cases are diagnosed by corneal scrapings stained with KOH, this method gives rapid results, but its sensitivity is low and it has a high false-positive rate. Also the procedure does not allow one to review the slides. Liesegang and Forster suggested a special stain, i.e. Glomeri methenamine silver(GMS), which has yielded 86-89% sensitivity compared with KOH stain's sensitivity of 25-33%.[4,5] Because the GMS staining technique is complicated, expensive and time-consuming to perform,[6] we studied another stain, the PAS stain, which appears to offer advantages similar to those of GMS stain and compared to KOH and Gram stain.

Subjects and Methods

Corneal scrapings were taken from 16 cases clinically suspected of having fungal keratitis; the scrapings were stained by KOH, Gram, and PAS stains. Specimens were also cultured for bacteria and fungi. Cultures for herpes simplex virus were performed in some cases. Corneal buttons obtained from cases who had undergone penetrating keratoplasty because of uncontrolled infection were sent for smear, re-culture and pathological examinations. For the PAS stain, the smears were fixed in 95% alcohol for five minutes and then oxidized with periodic acid for five minutes and rinsed in distilled water. Next, Coleman's Feulgen reagent was poured on the slide and left on it for 15 minutes. Afterwards they were washed in water for 10 minutes until they turned pink; they were then dehydrated in 95% alcohol (4 jars), absolute alcohol (2 jars) and xylene (2 jars), for a total of about 10 minutes. All cultures, KOH stain and Gram stain were reported by the laboratory microbiologists, and the PAS stain and corneal specimens were reported by the pathologists.

Results

Nine out of 16 cases were reported positive, for fungal keratitis and seven out of nine were cultured positive, as shown in Table 1. Based on cultures, the rest were reported to have bacteria or herpes simplex. Table 1 shows that, although some cases had negative culture for fungus, they were positive from smears. Thus, it is quite important to examine all smears carefully even though cultures are negative. Corneal scrapes with PAS stain reported more positive results compared with KOH or Gram stain and cultures as shown in Table 2. In addition, detection of fungus with PAS stain was easier (Fig. 1 and 2).

Table 1. Results of different laboratory techniques in detecting fungal keratitis.

Case No.	KOH	Gram	PAS	Culture	Corneal button after PK
1.	+	-	+	No growth	
2.	-	-	+	Curvularia	+
3.	-	-	-	Aspergillus	+
4.	-	-	+	Fusarium	
5.	-	-	+	Penicillium	+
6.	+	+	+	No growth	
7.	+	-	+	Curvularia	+
8.	-	-	+	Aspergillus	
9.	+	-	+	Penicillium	

Table 2. Comparison of positive reports of different methods used for detection of fungi.

	No. of cases	% sensitivity
KOH	4/9	44.44
Gram	1/9	11.11
PAS	8/9	88.89
Culture	7/9	77.78

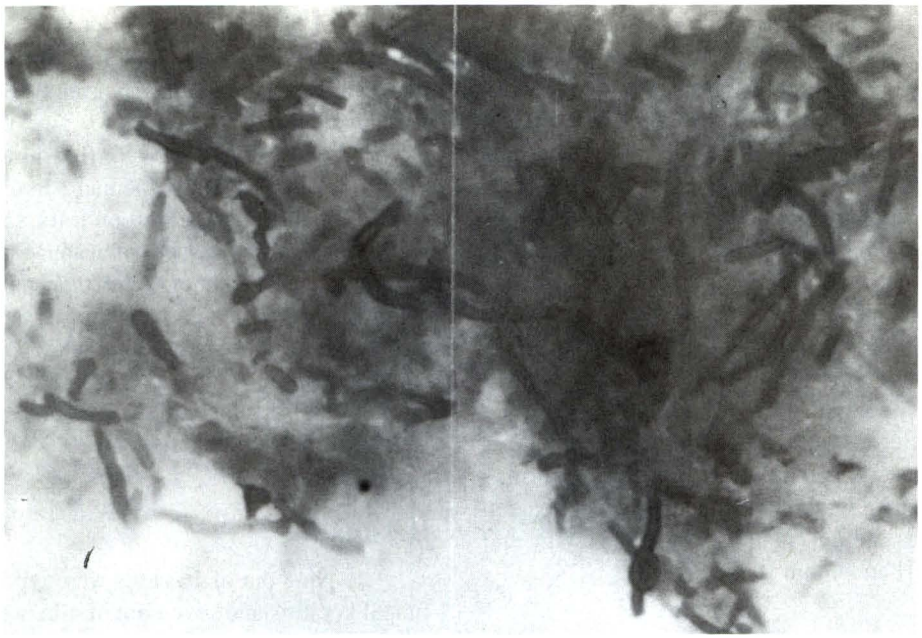

Figure 1. PAS stained corneal hyphae (x 400).

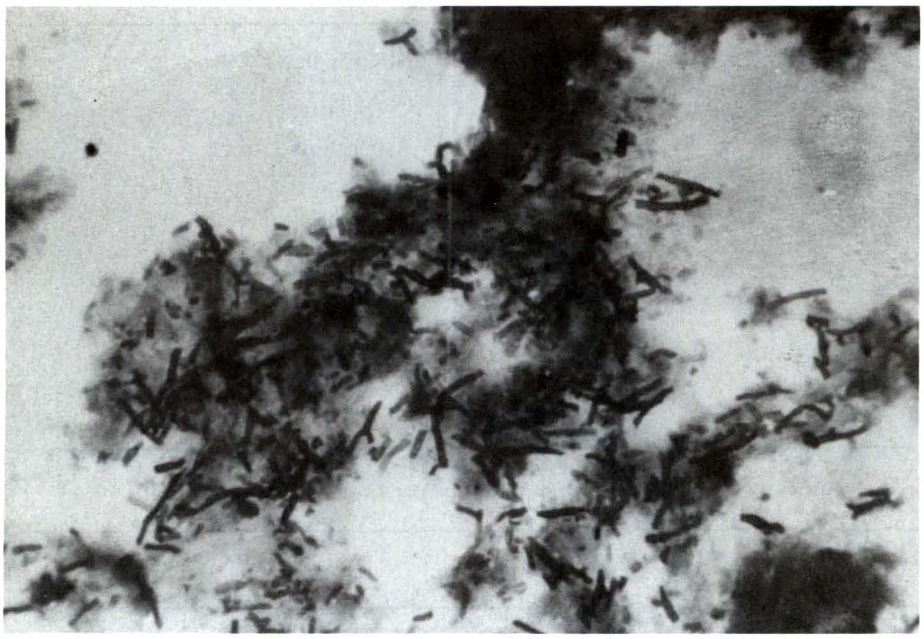

Figure 2. PAS stained corneal hyphae (x 100).

Discussion

We obtained good fungus-positive results by the PAS stain, i.e. 88.89% positive reports. The results were greater than those obtained with KOH and Gram stains. The PAS technique is quite simple and affordable. The time required to stain corneal scrapings is about 30-40 minutes which is shorter than the three hours required for the GMS stain. The detection of fungal septa with PAS is easier than with GMS stain. In addition, revision of the slides can be carried out.

KOH and Gram stain results are unreliable if the hyphae are small in number. Therefore, we suggest that PAS-stained corneal scrapings should be used in cases suspected of having fungal keratitis, as it is superior to KOH and Gram stain. In this stydy, two out of nine cases had negative cultures but positive PAS smears. This indicates the necessity of examining smears because they may contain fungi even though no growth is reported in the cultures.[7,8]

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