A comparative study of Chicago Sky Blue and Parker™ ink blue black potassium hidroxide in the diagnosis of dermatophytes

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Abstract

Parker ink blue black potassium hydroxide (KOH) is a regular stain for the diagnosis of dermatophytoses. KOH giving less color contrast to the fungal element. The Chicago Sky Blue (CSB) solution is a new contrast have various sensitivity and specificity values. This is analytical observational,cross sectional design study conducted at Dermatomycology Division of Outpatient Clinic, Dr. Soetomo General Hospital. 40 samples from lesion and perilesional infected area, diagnosed dermatophytoses with culture positive was include in this study. All two tests were performed examined by KOH and CSB. The slides were examined after incubation in x 10 and x 40 magnifications by experienced observer. The sensitivity, specificity and McNemar test of CSB and KOH was calculated using culture as the standard test. The sensitivities, specificities, PPV, NPV of the Chicago sky blue and KOH respectively are 95% and 100%, 95,24% and 100%, 100% and 100%, with McNemar test p = 1 and Kappa score is 0,950. The Chicago Sky Blue stain provides a good color contrast and IT could be a alternative staining for the dermatophytoses.

Introduction

Direct microscopic examination using KOH 10-30% is a simple and cheap examination that is widely used to diagnose dermatophytoses. The regular stain consist of Parke ink blue-black added at 10-30% KOH. An examination the fungal element is performed using a light microscope at x 10 and x 40 magnification. Fungal elements show hypha or arthrospora/arthroconidia. Though the fungal filaments did not take up the color of ink parker blue black-KOH stain and encountered of artifacts and precipitate.[1] Another disadvantage is requires special expertise of experienced analysts in viewing the visible fungal elements that appear transparant between the basal cell debris. This difficulty is probably caused by a change in the formulation of the Parker ink that it no longer provides the appropriate color contrast to the fungal elements.[2] This results in KOH often giving false negative results of 5% to 15% .[1]

Alternative of contrast stain can also be used to enhance the contrast of fungal elements such as acridine orange, Calcofluor white, or Blankophor. These stains require a fluorescence microscope with a suitable filter but not all small laboratories or dermatological examination have fluorescence microscopes.[3,4] Chicago Sky Blue (CSB) and black chlorazole are other alternative contrast. In a previous study conducted by Tambosis, 2012 in dermatophytoses patients, samples taken from skin and nail fungus infections, using CSB (CSB 1% + KOH 10%) compared the contrast staining of chlorazole black, and KOH 10% + Parker inks as control. The sensitivity and the specificity of CSB, chlorazole black, and KOH + Parker ink respectively were 78% and 96%; 63% and 97%; and 48% and 96% respectively. The sensitivity and specificity of CSB was higher than that of KOH.[5] Chicago sky blue stain provides a color contrast, making interpretation easy and very affordable. A 12 cc bottle of CSB stain costs $25 and is sufficient for 200 tests. We believe the new contrast CSB stain has the potential to replace the KOH wet mount as the routine method for the rapid diagnosis of superficial mycoses.[6]

Other study found different resut, in a large-scale study conducted by Liu in 2015 in dermatomycoses and onychomycosis patients with fungal cultures as the gold standard, a CSB stain was compared to KOH. Analyses using McNemar's test showed $p > 0.05$. Sensitivity, specificity, PPV and NPV between CSB and KOH stain on skin and nail specimens are similar, but using Kappa tests showed that KOH sensitivity was lower than CSB in skin specimens and total specimens (Kappa value <0.4). The sensitivity of CSB stain is superior than KOH.[7]

There is various of diagnostic result from some previous study so we conduct the diagnostic test of sensitivity and specificity of CSB compared to ink Parker™ blue-black KOH in dermatophytoses patients. In this study using CSB solution consist of KOH 10% + Chicago Sky Blue 6B 1%.

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Materials and Methods

This study was conducted in Dermatomycology Division of Outpatient Clinic, Dr. Soetomo Surabaya Hospital. Inclusion criteria were patients clinically diagnosed as dermatophytoses based on anamnesis, clinical examination, wood lamp examination, culture positive using sabourauds dextrose agar with chloramphenicol and cycloheximide were used for the growth of dermatophytes and incubated at 37^0 C for maximal 4 weeks. Exclusion criteria were patients with a history of using topical anti-fungal less than 2 weeks or history using oral anti-fungal less than 4 weeks. 40 sample from lesions and perilesion (1 - 1.5 cm from active lesion) of dermatophytoses patients. Each sample was divided into two parts and placed on sterile petri dish, one part for lesion and the other for perilesion speciemen. A part of speciemen from lesion and perilesion divided into fungal culture media fungal using sabouraud dextrose agar. Other speciemen also placed on four glass slides, two parts for lesional and

perilesional KOH examination and the ohters slide for lesional and perilesional CSB examination. One drop of 20% KOH + ink parker blue black was added to two slides followed by one drop of CSB stain to the other slides. Then all four slides were covered with coverslip and examined after inflamed and incubated in room temperature for 5 minutes under both x 10 and x 40 magnification. The KOH and CSB mounts were separately examined for the presence of yeast or hyphal elements by experienced observers.

Results

In this study specimens from skin and hair as shown in Table 1, no spesimen from nail. Several dermatophyte species found in this study were *Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton verrucosum, Micropsorum ferrugineum, Epidermophyton floccosum, Microsporum auduoinii.*

Most of fungal elements appeared as a distinct blue color using the Chicago blue-KOH stain. The Parker ink-KOH did not provide a good color contrast. Fungal filaments and yeast cells failed to take up the blue color and appeared as clear cells against a clear as seen in Table 2.

Based on the positive culture, KOH and CSB compared. The sensitivity, specificity, PPV, NPV value of KOH and CSB almost the same that resectively were 95% and 100%, 95,24% and 100%, 100% and 100% respectively, with a value of p=1, Kappa value is 0.950 (Table 3).

Disscussion

Direct microscopic examination ink parker™ blue black KOH 20% is a quick check, cheap, simple and requires minimal techniques. However KOH interpretation requires experienced analysts.[7] Culture is a gold standard has a high specificity and is the only routine confirmation test for identifying dermatophyt species.[1,2] Culture need long processing time. In this study only positive cultures were included, all samples were diagnosed as dermatophytoses. When ink parker™ blue black KOH 20% compared to culture, KOH has superior ability to detect fungal elements from clinical specimens. In this study KOH has a sensitivity value of 95.0%; specificity 100.0%; PPV 95.24%; NPV 100.0%, LH + can not be calculated, LH - 0,05 with Kappa value 0,950 (p=1). If CSB is compared with culture, sensitivity is 100.0%; specificity 100.0%; PPV 100.0%; NPV 100.0%; LH + can not be calculated, LH - 0,00 with Kappa value 1,000 (p=1). Fungal elements with CSB stain will be more easily recognizable with low microscope enlargement, and shorter screening times than using KOH stain.

Based on the Mcnemar test, in this study, the sensitivity, specificity, PPV, NPV of KOH and CSB are almost equal (p=1) with Kappa value > 0.950. The result of statistical analysis showed that ink Parker™ blue black KOH stain as well as CSB. No superior stain was obtained in this study despite CSB have higher sensitivity. Earlier study, Lim SC and Lim SL in 2008 comparing CSB and KOH + ink parker stain in dermatophytoses, and candidiasis, sensitivity and specificity of CSB higher than KOH 20%.[8] Tambosis E and Lim C comparing CSB, chlorazole black, and KOH in dermatophytoses and candidiasis revealed that CSB sensitivity was higher than other dyes.[5] The different result was obtained in a large-scale study by Liu in 2015 in dermatomycosis and onychomycosis patients with fungal cultures as the gold standard, CSB compared to control using KOH, using the McNemar test show the same sensitivity, specificity, PPV and NPV between CSB and KOH stain on skin and nail specimens (p > 0.05), but Kappa tests showed that the sensitivity KOH is lower than that of CSB in skin specimens and total specimens (Kappa value < 0.4). It is estimated that the sensitivity of CSB stain was superior to KOH.[7]

CSB-KOH stain can give more intense color than ink Parker™ blue black KOH. Fungal filaments did take up the color of CSB stain result blue color of fungal elements which is very contrast with the purple background as shown in Figure 1.

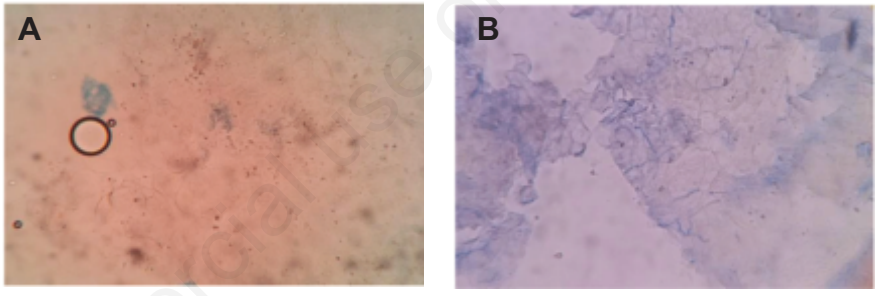

Figure 1. A. Ink ParkerTM blue black KOH stain, transparant hifae and precipitate; B. CSB stain, blue hifa without presipitate (X 400 magnification).

Table 1. Isolation of dermatophytes in infected area.

Dermatophytes	Skin	Hair	Nail
Trichophyton mentagrophytes	6	-	-
Trichophyton rubrum	6	-	-
Trichophyton verrucosum	1	-	-
Mrichophyton ferrugineum	2	1	-
Epidermophyton floccosum	3	-	-
Microsporum auduoinii	1	1	-

Table 2. Details distribution of Parker-ink KOH and CSB-KOH.

Variables	Lesion		Perilesion	
	KOH	CSB	KOH	CSB
Precipitate				
Precipitate (+)	7	0	5	0
Precipitate (-)	13	0	15	0

Table 3. Sensitivity (Sn), specifisity (Sp), *Positive predictive value* (PPV), *Negative Predictive Value* (NPV), Likelihood Ratio (LH) Parker-ink KOH 20% and CSB.

	Sn	Sp	PPV	NPV	LH+	LH-	Kappa	P value
KOH	95,00%	100,00%	100,00%	95,24%	NA	0,05	0,950	1
CSB	100,00%	100,00%	100,00%	100,00%	NA	0,00	1,000	1

Note: LH+: likelihood ratio possitive, LH- : likelihood ratio negative, NA: not available.

Tambosis E and Lim C, 2012 explains that ink Parker™ blue black KOH produce transparant fungal elements with brown background whereas CSB stain can provide better contrast.[5] Several previous studies, both performed by Lim CSH and Lim SL in 2008, Tambosis and Lim C, 2012; Fonseka S and colleagues, 2011 that comparing CSB and parker ink KOH 20% stain in dermatophytoses, PV, and candidiasis showed that CSB stain was well absorbed in all fungal elements, ink parker KOH only absorbed by Malassezia *spp.*[5,6,8] In addition, ink Parker™ blue black KOH presence of bluish precipitates in fungal elements. The KOH solution dissolves most of the cellular debris without affecting the chitin component of the cell wall of the fungus, so that many artifacts.[6] Bluish precipitate may also be due to differences in clinical material conditions, number, location, or shape of lesions, and also differences in ink Parker™ blue black KOH 20% reagent used in this study with prior. Aims of this study to perform diagnostic tests between ink Parker™ blue black KOH compare with CSB-KOH in dermatophytoses patients. The result of the analysis showed similarity with previous study.[7] The analys statistic of CSB-KOH has as good as Parker ink-KOH in this study.

Conclusions

The Chicago blue stain is provides a good color contrast, making interpretation of the stain easier. Fungal filaments stained as a distinct blue color against a purple or pale background. It is a suitable as confirmation test if Parker ink-KOH stain give provide negative result in suspect dermatophytoses case.

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