New Contrast Stain for the Rapid Diagnosis of Pityriasis Versicolor

Successful treatment of pityriasis versicolor requires an accurate diagnosis. Culture is not useful because its etiologic agent Malassezia furfur is part of the normal skin flora. Hence, diagnosis is usually based on direct microscopic examination of skin scrapings. The standard potassium hydroxide (KOH) wet mount suffers from a lack of color contrast. Parker blue-black ink has been added to potassium hydroxide to highlight fungal hyphae and spores against the surrounding cellular debris. The fluorescent brightener calcofluor white specifically binds to chitin, but this method incurs the additional cost of a fluorescence microscope. In this study, we compared a new contrast stain with the Parker-KOH stain in patients with a clinical diagnosis of pityriasis versicolor.

Methods. The study population comprised male and female patients who were diagnosed as having pityriasis versicolor by the dermatologist Kah-Beng Lim, MRCP(UK) (K.B.L.).

Areas of skin to be scraped were first cleaned with an alcohol swab to remove traces of creams and reduce surface bacteria. Skin scrapings were taken with a number 15 scalpel blade, placed on a clean microscope slide, and covered by a second microscope slide. The 2 slides were then bound at the ends with microporous tape, labeled with the patient’s name, and stored in a screw-capped plastic bottle for microscopic examination.

Duplicate slides were made of each patient’s skin scrapings for microscopic examination and coded by K.B.L. One slide was stained with the contrast stain and the other with the Parker-KOH stain containing 1 part Parker blue-black ink and 1 part 20% KOH. Both investigators collaborated in the reading of the slides but were not aware of all other test results when they read the slides. The Parker-KOH stain was used as the reference method for calculating the sensitivity and specificity of the contrast stain.

Contrast Stain. The new contrast stain contains 1% Chicago sky blue 6B and 8% KOH as the clearing agent. A drop of the contrast stain was mixed into the specimen on a clean microscope slide and left to sit in a humidifying chamber (covered plastic container lined by moist paper towel) for about 20 minutes at room temperature. A coverslip was applied over the specimen and gently pressed to remove air bubbles. Excess stain was blotted off with a paper towel. The slide was first scanned at an original magnification of ×10 using an Olympus CH-2 microscope (Olympus, Tokyo, Japan). Fungal elements stained blue against a purplish background of cellular debris. Malassezia furfur was confirmed at an original magnification of ×40 on the finding of short angular hyphae and spherical spores. Oil immersion (original magnification ×100) was used for a more detailed study of fungal morphology.

Parker-KOH Stain. Briefly, a drop of the stain was mixed into the specimen, covered with a coverslip, and left to sit at room temperature for 20 minutes. The coverslip was then pressed gently to remove air bubbles and excess solution was blotted off with a paper towel. Slides were examined at original magnifications of ×10 and ×40 with the Olympus CH-2 microscope. Malassezia furfur was confirmed by the presence of blue staining hyphae and spherical spores. The sensitivity and specificity of the new contrast stain were calculated using 2 × 2 contingency tables with the Parker-KOH stain as the reference method.

Results. A total of 24 specimens were examined, 22 (92%) of which were positive for M furfur by both methods. The new contrast stain had a sensitivity and specificity of 100%, using the Parker-KOH stain as the reference method. Malassezia furfur cell walls stained blue against the purplish background of cellular debris. Hyphae were short and angular, and there were often clusters of spherical or flask-shaped yeasts, giving the appearance of spaghetti and meatballs (Figure 1). Darker staining was observed when the slide was left to sit longer. Hyphae and spores appeared blue against a light orange background of cellular debris with the Parker-KOH stain, and bluish precipitation was noted (Figure 2).
Analysis and interpretation of data: magnification of spores against orange cellular debris (a) and bluish precipitate (b) (original magnification ×400).

Comment. Both investigators are novices to microscopic examination of skin scrapings. Hence, it was decided that they could confer in the interpretation of the slides. The new contrast stain achieved a sensitivity and specificity of 100% when compared with the Parker-KOH stain as the reference method. A bluish precipitate was commonly observed with the Parker-KOH stain, whereas none occurred with the new contrast stain. In addition, the new stain is better at highlighting the morphologic features of both spores and hyphae compared with the Parker-KOH stain. Budding M furfur yeasts with septa at the neck can be clearly seen under oil illumination. The new contrast stain is therefore a useful alternative to the Parker-KOH stain. Unpublished results (January 6, 2008) from an ongoing study by the authors suggest that this stain may also be very useful for the diagnosis of dermatophytosis.

The new contrast stain contains KOH as the clearing agent. Laboratory overalls and gloves should be worn to protect clothes and hands. The staining procedure is simple and rapid to perform and requires only an ordinary light microscope.

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Author Contributions: Drs S.-L. Lim and C. S.-H. Lim had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Both authors contributed equally to the study. Study concept and design: S.-L. Lim and C. S.-H. Lim. Acquisition of data: S.-L. Lim and C. S.-H. Lim. Analysis and interpretation of data: S.-L. Lim and C. S.-H. Lim. Drafting of the manuscript: S.-L. Lim and C. S.-H. Lim. Critical revision of the manuscript for important intellectual content: S.-L. Lim and C. S.-H. Lim. Administrative, technical, and material support: S.-L. Lim and C. S.-H. Lim.

Financial Disclosure: None reported.

Additional Contributions: Kah-Beng Lim, MRCP(UK), who provided the new contrast stain used in this study, is the father of the investigators. He collected and coded the specimens and advised on the study design but was not involved in its evaluation.


Late Cutaneous Manifestations 14 to 20 Years After Wartime Exposure to Sulfur Mustard Gas: A Long-term Investigation

Sulfur mustard gas (SM) is a potent alkylating agent that has a long history of use as a chemical warfare agent, including recent use by Iraq against Iranian soldiers and civilians. The organs most commonly affected by SM are the skin, eyes, and airways. Skin lesions are seen in more than 90% of the patients exposed to SM. Although the acute systemic and cutaneous effects of SM are well known, few investigations have dealt with the long-term effects. The aim of this study was to investigate the long-term cutaneous problems experienced by survivors of SM attack several years after exposure.

Methods. The 800 male subjects of this cross-sectional descriptive study were recruited from surviving veterans of the Iraq-Iran war (1) whose exposure to SM from 1983 to 1988 was documented in their wartime medical records and (2) who had at least 1 cutaneous sign or symptom at the time of evaluation for this study. The survey was performed 14 to 20 years after exposure of the subjects to SM. Physical examinations conducted for this investigation focused on defining the diagnosis and the extent of the cutaneous disorders among the subjects and their correlation with previous SM exposure. Laboratory investigations including histopathologic studies were carried out whenever clinically indicated.

The clinical data were collected by the cluster sampling method. The frequency of each clinical sign or disease was calculated with 95% confidence intervals, and the final data were statistically analyzed and compared with some available related data in the healthy population using the Fisher exact test and SPSS software, version 12 (SPSS Inc, Chicago, Illinois).

Results. The mean (SD) age of the patients was 39.3 (9.8) years (age range, 18-80 years). Almost all of the patients were exposed to SM only once.

The cutaneous signs and disorders could be categorized into 3 different groups (Table). The first group was nonspecific skin disorders (93.4%), including seborrheic dermatitis, eczema, multiple cherry angiomata, vitiligo, and tinea versicolor in study subjects exposed to SM compared with the fre-