The Myanmar Health Sciences Research Journal, Vol. 17, No. 1, 2005

SHORT REPORT

Evaluation of microscopy for detection of acid-fast bacilli from sputum after overnight treatment with sodium hypochloride

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Microscopy of direct sputum smears for acid-fast bacilli (AFB) remains the gold standard for diagnosis of tuberculosis (TB) in most laboratories of developing countries. The specificity of positive acid-fast smear is high [99.3% to 99.9%] but the sensitivity is low ranging from 22% to 78% [1]. Previous studies showed that the sensitivity of AFB smear is improved by digestion of sputum with 5% sodium hypochlorite (NaOCl) or household bleach and concentration of bacteria by centrifugation [2,3,4].

However, this method needs access to a centrifuge and it may cause major inconvenience for microscopic centers in remote areas with no electricity and limited laboratory facilities. Thus, this study was carried out to detect whether sedimentation of NaOCl treated sputum by overnight standing at room temperature could replace the step of centrifugation.

Three hundred and seventy eight sputum samples were collected from tuberculosis patients attending TB Centre, Yangon. Slides for direct smears were prepared from purulent part of sputum and stained by Ziehl-Neelsen method for direct microscopy.

The remaining sputum was divided into two parts and each was transferred to 10ml screw capped tube and mixed with equal volume of 5% NaOCl. The mixture was incubated at room temperature for 10 minutes. Then 6-8 mililitre of distilled water were added to each sample and one tube was centrifuged at 3000g for 15-20 minutes. After discarding the supernatant, the sediments were stained by Ziehl-Neelsen method for microscopic examination.

The other tube was allowed to stand at room temperature overnight after adding distilled water. Then the next morning the supernatant was discarded and the sediments were stained by Ziehl-Neelsen staining and examined under ordinary light microscope.

Out of 378 sputum samples,110 samples were positive by direct smear microscopy, 122 samples were positive by NaOCl-centrifugation and 109 samples were positive by NaOCl-overnight standing. It was found that there is no improvement in sputum smear microscopy of AFB by NaOCl-overnight standing method and the smear results can be obtained only on the next day. Almost all direct smear positive samples were also positive in NaOCl-overnight standing (only one slide showed negative with NaOCl-overnight standing method but that sputum sample had insufficient amount) and all NaOCl treated sputum samples showed no putrefaction on the next day at room temperature.

As NaOCl is a disinfectant, it can be used safely in condition where the sputum samples have to be kept for some times at

room temperature with lower risk of laboratory infection without interfering the positive rate of direct microscopy. But it cannot be applied to samples intended for culture because NaOCl kills mycobacteria. In our method, we add 6-8 millilitre of distilled water to the sputum after treating with equal volume of 5% NaOCl for 10 minutes. Therefore, disintegration of bacilli by acting too long with NaOCl did not have much effect in our method, as the morphology and average number of AFB seen per microscopic field were not quite different from direct smear microscopy.

Finally, it was concluded that sedimentation of NaOCl treated sputum by overnight standing could not replace the step for centrifugation for improvement of smear sensitivity. However, it could be used in remote microscopic center with no access of centrifuge and refrigerator when sputum specimens have to be collected late in the evening and must be kept as such at room temperature until tomorrow morning for direct microscopic examination. Moreover, this method reduces the risk of laboratory infection and putrefaction of the sputum specimens.

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