A Comparative Study of Two Staining Methods For The Detection of Tuberculosis Bacilli (TB)

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Abstract

The presence of acid fast bacilli in sputum specimen was investigated comparatively with Ziehl-Neelsen (ZN) and Kinyoun Cold staining (KC) in order to determine sensitivity in detecting Tuberculosis Bacilli (T.B.). This study was employed on patients with severe lower respiratory tract infection to compare between these two stains. Which revealed that Ziehl-Neelsen procedure gives better results than Kinyoun cold procedure.

Introduction

Tuberculosis is still rampant in tropical countries, and, it is one of the most important causes of human death worldwide, although the disease has receded in technically advanced countries of the world (1). Over the last thirty years, the absolute number of tuberculosis patients throughout the world is still increasing, owing to population growth in the more underprivileged population (2). More than 20,000 new cases of tuberculosis and over 12,000 deaths are reported annually (3). Most cases are caused by Mycobacterium tuberculosis, acquired from other humans via the respiratory route. Once in the lungs, the bacteria grow and eventually are surrounded by lymphocytes, macrophages, and connective tissue in a hypersensitivity response that forms a hard nodule called a tubercle. The disease process usually stops at this stage, but the bacteria remain alive within the tubercle (4, 5).

The main characters of Mycobacterium tuberculosis are poorly staining gram positive, which may be slightly curved, but are slender. Variable in size, they are approximately 3μ x 0.3μ. Typically they are acid-alcohol fast due to the waxy material in their cell walls. (6,7,8).

Materials And Methods

This study was conducted on 183 patients with lower respiratory tract infection and clinical suspicion of tuberculosis in Al-Zahrawi Teaching Hospital in Mosul, November, 2005 – May, 2006. Two staining assays used for the detection of Mycobacterium tuberculosis. Technique performed was: sputum examination by staining smear of sputum, first assay used Ziehl-Neelsen stain and the second utilized Kinyoun Cold stain (9,10).

Results

The culture was employed as the reference method. Forty patients (21.8%) were diagnosed as having T.B. by culture. Compared with culture, ZN stain and Kinyoun Cold stain had sensitivities of 67%(27/40) and 37.5%(15/40) respectively. On the other hand, the typical appearance of acid fast bacilli is more clearness by using (ZN) comparing with (KC) as shown in photograph 1 & 2, which represent the smear of the sputum from the same patient stained by ZN and KC.
Photograph 1: Film of concentrated specimen of sputum stained by the Ziehl-Neelsen method X1000

Photograph 2: Film of concentrated specimen of sputum stained by the Kinyoun Cold method X1000

Discussion

M. tuberculosis is not a commensal, and the demonstration of the typical acid alcohol-fast bacilli cultured from a specimen is presumptive evidence of infection (11,12). There are different stains using for the diagnosis of tuberculosis such as ZN, KC and fluorescence microscopy (FM) staining (13, 14, 15). This present report has compared between ZN and KC staining. Study data indicate that in the diagnosis of TB, ZN has greater sensitivity than KC. In particular, in the case of a single specimen,
the diagnosis value of ZN is quite significant. It is, therefore, possible to conclude that both KC and ZN staining can be used for the diagnosis of TB when there are more than specimens. However if only one or two specimens are available ZN staining is preferable, also it is beneficial for diagnosis especially in negative cases or suspicious cases of tuberculosis. This may be due to the use of heat with (ZN) but not with (KC), which revealed the importance of heat in the clearness of staining.

An acid fast staining can detect mycobacteria in clinical specimens rapidly and specifically. It equally stains living and dead bacteria. It would be of more clinical use if the viability of mycobacteria in a sample was determined by the staining such as fluorescent which detect live or dead bacteria (16, 17, 18, 19). Some studies have demonstrated that acid-fast smear using greater than or equal to 5ml of sputum increases sensitivity for M. tuberculosis and accelerates treatment of T.B (20).

The rising incidence of tuberculosis worldwide means an increasing burden on diagnostic facilities, so tests simpler than Ziehl-Neelsen staining are needed (21,22,23). The researcher Arias-Bouda developing Enzyme linked immunosorbent assay for detection of lipoarabinomannan (LAM) in human sputum samples (24), and others use polymerase chain reaction (PCR) assay for rapid detection and identification (25,26,27), and ligase chain reaction as primary screening tool for the detection of culture positive tuberculosis (28). We recommended that further studies on the extrapulmonary tuberculosis and study the presence of acid fast bacilli in multiple specimens.

References


