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Ziehl-Neelsen Stain versus Tuberculosis Rapid Test in the Laboratory Diagnosis of Pulmonary Tuberculosis

Layla S. Abd Al Aziz

Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan

Abstract

Background: Correct diagnosis and optimum treatment are considered effective control measures. Many serological tests had been employed in the diagnosis of tuberculosis including radioimmunoassay assay (RIA) and latex agglutination. Both tests are neither sensitive nor specific. Enzyme-linked immunosorbent assay (ELISA) can be used to detect mycobacterial antigen in sputum. Culture techniques can be used to isolate *Mycobacterium tuberculosis* (*Myco. tuberculosis*) but it is a tedious procedure and time-consuming.

Objective: To compare the efficiency of the Ziehl-Neelsen (ZN) stain and the tuberculosis rapid test (TRT) in the laboratory diagnosis of pulmonary tuberculosis.

Materials and methods: A total of 80 patients suspected of having pulmonary tuberculosis were studied. Sputum and finger-prick blood samples were collected from each patient and examined for the presence of *Mycobacterium tuberculosis* using the Ziehl-Neelsen (ZN) stain and the tuberculosis rapid test (TRT).

Results: ZN staining method demonstrated 75 (93.8%) positive cases, and TRT showed 22 (27.5%) positive cases. The ZN stain was found more sensitive (93.8%) than the TRT (27.5%). Males were found fairly more affected than females. The age group 26-36 years was the most susceptible age group to tuberculosis (TB) infection.

Conclusion: The ZN stain technique was more sensitive, and the TRT was not suitable for screening suspected cases of pulmonary tuberculosis.

Key words: Ziehl-Neelsen Stain, Tuberculosis rapid test, Pulmonary tuberculosis

Introduction:

Tuberculosis is one of the most serious infections of the developing world. It affects apparently healthy as well as immunocompromised persons. Tuberculosis affects mammalian, avian and

cold blooded animals. Mammalian tuberculosis may be divided into human, bovine and vole types. The human type is responsible for pulmonary tuberculosis in adults. The bovine type may occasionally causes also the pulmonary from[1].

Tuberculosis (TB) remains as one of the major health problems and a leading cause of morbidity and mortality around the world. It is likely to remain one of the 10 most important causes of premature mortality worldwide in the coming two decades. Tuberculosis is more common in elderly, chronically malnourished, alcoholic, poor and homeless people. Nearly two billion people are infected with tuberculosis worldwide; 31 millions of them are HIV infected patients. TB morbidity has doubled during the last 10 years. In1993 World Health Organization (WHO) declared tuberculosis as a global emergency in humans[2].

Tuberculosis kills more people than any other infectious disease. The incidence of the disease has continued to rise by over 7 million each year in developing countries, and around 4 million die from it. The most infected are young adult and children in South East Asia, Sub-Saharan Africa, Latin America, and West Pacific countries. Factors contributing to resurgence of tuberculosis in developing countries are problems of drug resistance, inadequate treatment, continuing poverty, malnutrition, overcrowding, armed conflicts, and increasing numbers of displaced persons. Infection with HIV greatly increases the risk of contracting tuberculosis and accelerates its progress. WHO estimates that tuberculosis causes up to 40% death in Africa and South East Asia[3].

More sensitive and specific tests such as polymerase chain reaction are too expensive for routine laboratory diagnosis of TB. Therefore direct microscopy examination of sputum became the method of choice in diagnosing pulmonary tuberculosis. Recently introduced is the tuberculosis rapid test to detect antibodies in serum of pulmonary TB patients. It is considered a reliable test to give an early diagnosis of TB.

Materials and methods

This was a qualitative study conducted to compare the efficiency of the Ziehl-Neelsen stain and the tuberculosis rapid test in the laboratory diagnosis of pulmonary tuberculosis. It was a descriptive, cross-sectional, and laboratory case study. The study was carried out at Port Sudan Specialized Teaching Chest Hospital (Sudan) during the period from April to July 2010. Sampling was a non-probability random, convenience type. The sampling population frame was covering Sudanese males and females aged 15-80 years living in Port Sudan area. Sample size was 80 samples. Inclusion criteria were pulmonary tuberculosis patients with typical clinical symptoms of tuberculosis. Exclusion criteria were patients aged less than 15 and more than 80 years.

Approval to conduct this study was taken from the Ethical Committee of the Faculty of Medical Laboratory Science (Al Neelain University, Khartoum, Sudan). Verbal consent was obtained from all patients enrolled in this study. Data was collected using a structural interview questionnaire. Demographical information (name, age, and gender) was recorded. Data was analyzed by the Statistical Package for Social Studies (SPSS) program.

Three consecutive sputum specimens were collected from each patient in plastic, clean, dry, wide-mouth, screw capped containers to avoid leaks and aerosol formation. Patients were advised to collect early morning sputum samples prior to mouth washing, and following deep cough to produce a real sputum and not mere saliva. The containers were labeled by patients'

names, serial numbers, and dates of collection. The purulent part of each sputum sample was selected and picked up by applicator wood sticks and applied onto a microscope slide. The smear prepared was neither thin nor thick. Smears were left to dry and then fixed by 70% alcohol. Control smears from known tuberculosis patients were included as positive controls. In addition, smears from known, non-tuberculosis patients were included as negative controls. ZN stains were performed by placing the smear slides on the staining rack and covered with carbol fuchsin as a primary stain. The slides were heated until vapor rises and left for 5 minutes. The slides were washed by distilled water until all deposit disappears. Then the decolourizer (3% acid-alcohol) was added for 2 minutes. The slides were washed, counter-stained by malachite green, and then washed with distilled water until no stain was visualized and no deposit remained. The slides were air-dried. Positive and negative control smears were prepared simultaneously as above. The slides were examined by a light microscope, oil immersion objective lens (X100). Acid-alcohol fast bacilli were viewed as bright red against a green background.
The result was interprtated according to Wagne (1995)[4] as follows:
a) No acid fast bacilli per 100 fields: negative
b) 1-9 acid fast bacilli per 100 fields: scantly
c) 10-100 acid fast bacilli per 100 fields: +
d) 1-10 acid fast bacilli per one field: ++
e) More than 10 acid fast bacilli per one field: +++.

The tuberculosis rapid test was performed first by washing the patient's hand with soap and warm water and cleaned with an alcohol swab. After drying, the hand was massaged without touching the puncture site by rubbing down towards the finger tip of the middle finger. Then the skin was punctured with a sterile lancet, the first sign of blood was wiped away, and the hand was gently rubbed from wrist to palm and finger to form a rounded drop of blood over the puncture site. The patient's finger was positioned so that the drop of blood was just above the specimen well (S) of the test device. 3 hanging drops of the finger whole blood (approximately 75 µL) were allowed to fall into the center of specimen well (S) on the test device. Then one drop of buffer was added, and the timer was started. Touching the finger directly to the specimen well (S) was avoided. When the colored lines appear, the result was read within 10 minutes.
Result was interprtated as follows:
a) Positive: Two distinct colored lines appear: One line in the control region (C) and another line in the test region (T)
b) Negative: One colored line appears in the control region (C), and no apparent colored line in the test region (T)
c) Invalid: Control line fails to appear neither in the control region (C) nor in test region (T).

Results

Sputum samples were collected from 80 patients suspected of having pulmonary tuberculosis and examined for presence of *Myco. tuberculosis* by using the ZN stain and the tuberculosis rapid test. The ZN stain showed 75 positive smears (93.7 %), while the tuberculosis rapid test showed 22 positive smears (27. 5 %) as shown in Table (I).

Table (I): Results of the ZN stain and the TRT

Result	ZN stain		TRT	
	No.	Percent	No.	Percent
Positive	75	93.8	22	27.5
Negative	5	6.2	58	72.5
Total	80	100	80	100

Statistically this difference is significant ($p = <0.05$). The ZN stain gave more positive results than the tuberculosis rapid test suggesting that it was more sensitive than the latter.
A total of 80 patients (males and female) were enrolled in this study, 54 of them (67.5%) were males, and 26 (32.5%) were females. 49 males (61.3%) and 26 females (32.5%) were positive by the Z.N stain, while 16 males (20.7%) and 6 females (7.5%) were positive by the tuberculosis rapid test (Table II).

Table (II): Results of the ZN stain and the TRT according to gender

Gender	ZN stain		TRT		Total
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
Males	49 (61.3%)	5 (6.3%)	16 (20.7%)	38 (47.5%)	54 (67.5%)
Females	26 (32.5%)	0 (0.0%)	6 (7.5%)	20 (25%)	26 (32.5%)
Total	75 (93.8%)	5 (6.3%)	22 (27.5%)	58 (72.5%)	80 (100%)

Patients studied were categorized into various age groups. The age group 26-36 years gave the highest positivity (28%) for *Myco. tuberculosis* when tested by the ZN stain; and also gave the highest positivity (36.3%) for *Myco. tuberculosis* when tested by the TRT (Table III).

Discussion:

In this study the ZN stain was used to examine a total of 80 sputum samples; and the tuberculosis rapid test was employed to examine 80 finger-prick whole blood samples collected from patients suspected of having pulmonary tuberculosis. Each of the two methods had been found to have advantages and drawbacks.
The ZN stain was found to be specific, sensitive, and inexpensive. Its reagents are available and the morphology of *Myco. tuberculosis* can be directly studied. However its procedure is a bit tedious since at least three consecutive sputum specimens and 100 fields of each smear had to be examined before reporting the smear as negative, i.e. time-consuming. In addition, it requires careful heating to make the carbol fuchsin dye penetrate the bacilli, and overheating would damage the smear.

Table III: Results of the ZN stain and the TRT according to age incidence

Age groups (years)	ZN stain		TRT	
	Positive	Percent	Positive	Percent
15-25	11	14.7	3	13.7
26-36	21	28.0	8	36.3
37-47	11	14.7	1	4.5
48-58	10	13.3	3	13..7
59-69	14	18.6	5	22.8
70-80	8	10.7	2	9.0
Total	75	100.0	22	100.0

Tuberculosis rapid test, on the other hand, has a quick procedure, easy to perform, specific, and requires no equipment. However, it is less sensitive, and more expensive. The present study showed that young adults were fairly susceptible to *Myco. tuberculosis* (Table III). These findings were similar to those obtained in 1997 by Hamza and Majzoub[5].

The WHO had reported in 2002 that tuberculosis infection was more common in the age range 23-48 years[3]. In this context, the high incidence of tuberculosis (66. 3 %) was also prevalent in this age range (Table III).

The study findings also showed that males were fairly more susceptible to the disease than females (Table II). This same result was also reported by Hamza and Majzoub[5].

It may not be possible to adopt the tuberculosis rapid test as a routine diagnostic tool for pulmonary tuberculosis infection, not only because it is more expensive, but also because it is less sensitive (29.3%) than the ZN stain technique (93.8%).

Further studies are recommended to assess the sensitivity and specificity of the ZN stain and the tuberculosis rapid test. ZN stain is recommended for the microscopical detection of *Myco. tuberculosis* and the routine diagnosis of pulmonary tuberculosis.

Conclusion: The ZN stain technique was more sensitive than the tuberculosis rapid test. Pulmonary tuberculosis was fairly more common among males than females; and its incidence is higher among young adults. Tuberculosis rapid test is not suitable for screening suspected cases of pulmonary tuberculosis.

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