Confirmation of positive acid fast bacilli samples by tuberculosis bacilli culture.

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الملخص

هذه الدراسة هي محاولة لتقدير كفاءة تقنية صبغة Acid Fast Bacilli (AFB) في تشخيص التدرن في عينات مختلفة. انجزت هذه الدراسة في مستشفى الديوانية العام وقسم الاحياء المجهرية في كلية الطب, جامعة القادسية بين تشرين الاول 2005 الى تشرين الاول 2008. اخذ 1654 عينة سريرية من 750 مريض واجري عليها فحص صيغة AFB , وكانت 605 قشع,67 غسل قصب ,42 سائل غشاء الجنب , 4 ادرار ,7 سائل زاهد , و3 سائل شوكي .العينات الموجبة للصبغة AFB كانت 5.3 % موزعة على عينات مختلفة . خمسة وسبعون 75 مريض قيموا بواسطة الزرع على وسط Lowenstein Jensen وتعتبر كطريقة قياسية لتشخيص التدرن , من هذه 58 ثمانية وخمسون نموذج سجلت نتيجة موجبة للزرع . الحساسية لطريقة الصبغة زيل نيلسين والزرع كانت 68.9 % و 95.2 % على التوالي . التخصص لصبغة زيل نيلسن والزرع كانت 88.2 % و 45.4 % على التوالي . دقة التشخيص لطريقة صبغة زيل نيلسن والزرع كانت 73.3% و 73.3 % على التوالي . قيمة التنبؤ الموجبة لطريقة الصبغة والزرع كانت 95.5 % و68.9% بينما كانت قيمة التنبؤ السالبة 45.5 % و88.2 % على التوالي.

Abstract

This study attempts to estimate the efficiency of acid fast bacilli(AFB) stain technique to diagnosis tuberculosis in the different samples. This study was performed in general Al-Diawynia hospital and Department of Microbiology, Medicine College, Al-Qadisiha University between October 2005 to October 2008. A total of 1654 clinical samples from 750 patients analyzed for acid-fast staining. These were 605 sputum, 67 bronchial wash, 42 pleural fluids, 4 urine, 7 ascetic fluids and 3 cerebrospinal fluids. The stained smear-positive were (5.3 %) distributed in different samples .Seventy five patients (10 %) were evaluated by Lowenstein-Jensen (LJ) medium culture that it was employed as gold standard for tuberculosis diagnosis, of these 58 were culture-positive specimens. The sensitivity of Ziehl-Neelsen (ZN) stain Methods and LJ culture were (68.9 %) and (95.2 %) respectively. The specificity of ZN stain Methods and LJ culture were (88.2 %) and (45.4 %) respectively. The diagnosis accuracy of ZN stain Methods and LJ culture were (73.3 %) and (73.3 %) respectively. The positive predictive value of ZN stain Methods and LJ culture were (95.2 %) and (68.9 %), while negative predictive value were (45.4 %) and (88.2 %) respectively.

Introduction

Mycobacterium tuberculosis **is the most common cause of tuberculosis (T.B.) which is humanity's greatest killer which is out of control in many parts of the world. The disease is preventable and curable but it has been grossly neglected and no country is immune to it. The diagnosis of TB infection is vital both clinically and epidemiologically (1). Although growth of *M. tuberculosis* is slow on selective media but consider as standard Methods for other diagnostic tests. The microscopic detection of directly stained bacilli on the smear sample processed for clinical samples could provide quick results, but it is stay problematic due to specificity and sensitivity is not completely enough. Acid fast bacilli (A.F.B.) stain technique is critical for the respiratory physician and public health officer. The clinical presentation of extra-pulmonary T.B. is not typical, also A.F.B. stain are even less sensitive to fluid aspirates and tissue biopsies than sputum samples. The detection of A.F.B. in stained smears examined the presence mycobacteria in a clinical sample (2). It is the easiest and quickest procedure that can be performed, and it provides the physician with a preliminary confirmation of the diagnosis, also because it gives quantities estimation of the number of bacilli being excreted, the smear is vital clinical and epidemiologic importance in assessing the patient's infectiousness. Smears may be prepared directly from clinical specimen or concentrated preparation (3).**

The definitive diagnosis of tuberculosis depends on the isolation and identification of *M. tuberculosis*. The inoculation of concentrated bacilli from processed clinical specimens on solid media is a standard approach for confirmation of tuberculosis (4, 5). Culture Methods are more sensitive than microscopy as it can detect 10-100 mycobacteria per ml of sample and give positive result (6). Therefore culture is deemed to be the gold standard for diagnosis of TB. Despite its enhanced sensitivity and specificity, culture is of impractical clinical use, because it is costly, time consuming and requires specialized safety laboratories, which is usually not performed in most low income countries (7,8).In sputum smear microscopy, Z.N.(Ziehl- Neelsen) is the most commonly used technique, because of its simplicity and low cost. There are also other staining techniques for detection of acid fast bacilli, which are simpler, rapid and more sensitive than Z.N. (9).

Materials and Methods

Specimens: Seven hundred and fifty clinical specimens received for routine mycobacterial staining were processed between October 2005 to October 2008 in Al-Diawynia city. These were 605 sputum, 67 bronchial wash , 42 pleural fluid , 16 wound abscess, 6 peritoneal fluid, 4 urine, 7 ascetic fluid and 3 C.S.F. A total of75 samples were selected for culture Methods .Ziehl-Neelsen Stain: The procedure was that described by WHO laboratory guidelines (10). The fixed smears were flooded with the solution of 1% carbol fuchsine prepared by dissolving 1g of basic fuchsine in 10 ml of ethanol; this solution was diluted to 100 ml with aqueous 5 percent phenol. The smears were heated underneath until vapor start rising and were allowed to stand for 5 minutes. The smears were then rinsed with water and decolorized with 3 percent acid alcohol for 3 minutes. The slides were rinsed thoroughly with water and counterstained 0.1 percent malachite green solution and were let to stand for 1 minute. The slides were flooded with water and were allowed to air dry. The slides were then examined under microscope in x100 oil immersion.

Culture and Identification: Single slope slant per specimen were inoculated each with one 4 mm loopful of the centrifuged sediment, distributed over the surface. All cultures were incubated at 37 ^{o}C until growth was observed and those tubes in which growth was not observed after 8 weeks were regarded as negative were discarded. All cultures were examined after 48-72 hours after inoculation to detect any contaminants. Thereafter cultures were examined on 7th day for rapid growers once weekly thereafter, up to 8 weeks, for slow growers after which a definitive result was obtained. Typical colonies of *M. tuberculosis* were rough, tough, crumbly, waxy, non-pigmented (buff colored) and slow-growers (growth appeared after 2-3 weeks after inoculation). Growth of mycobacteria was confirmed by typical colony morphology and microscopy for A.F.B.

Statistical analysis: The specificity, sensitivity and diagnostic accuracy of the results were calculated by using statistical product and service solution, positive, negative predictive values and diagnostic accuracy also calculated (11).

Results and Discussion

The present study evaluated two diagnostic techniques used in the diagnosis of pulmonary and extra pulmonary tuberculosis. The staining technique was Z.N. stain and culture on L.J. medium, employed as 'gold standard'. In the study group 59% (n=440) were males and 41% (n=310) were females. A total of 750 pulmonary and extra pulmonary specimens were examined by A.F.B. stain (Z.N.) included in the study. Of these (75) 10% were evaluated by L.J. culture media.

From the total number only 5.3 % of the specimens were A.F.B. stain positive distributed in different samples; of them 72.5 % were sputum samples and the remaining all other samples. Of the 58 culture-positive specimens, 27 samples were positive for Z.N. stain, while the 19 samples were negative; of these 46 were sputum samples and the 12 from other samples (Table 1).

The added advantage of sputum smear microscopy is that it has very close relation with infectiousness: patient who are sputum smear positive and culture positive are far more likely to be infectious than culture positive but smear negative (12). The usual staining laboratory technique for staining A.F.B. used worldwide has been the Z.N. Methods . However the Methods requires controlled heating for its success, and there are certain disadvantages, e.g. multistage staining, a cumbersome heating procedure and the discomfort caused by aerosols of phenol. There are several modified staining techniques for detection of A.F.B. in sputum. The success of staining techniques depends on the ability of the dye to penetrate uniformly the cell wall of tubercle bacilli through their surface coated with waxy substance (13).

Table -1: distribution of specimens for three successive years.

Samples	No. tested	AFB positive		L.J. culture media positive	
		No.	%	No.	%
Sputum	605	29	4.8	46	7.6
Bronchial washing	67	7	10.4	7	10.4
Pleural fluid	42	3	14	3	14
Peritoneal fluid	6	_	0.0	-	0.0
Ascetic fluid	7	_	0.0	-	0.0
Urine	4	_	0.0	-	0.0
Wound abscess	16	1	6.2	1	6.2
CSF	3	_	0.0	1	33.3
Total	750	40	5.3	58	7.7

In the present study, maximum numbers of T.B. cases were observed in the economically most productive age group 40-49 and 50-59 years were positive for both A.F.B. stain and L.J-culture media (Table 2). T.B. was not diagnosed in the PTB suspects below 10 years; the reason behind this may be that purulent sputum is not available from children. This finding is in accordance to the previous report (14).This documented finding is consistent with another study by (15),who found that children older than 10 years were low infected with T.B. Regarding the age incidence it was found that the majority of infections occur in the young and middle age groups (40-60 years) more than other age groups (Table 2).

Table -2: Age distribution of smear and culture - positive.

Age group (year)	No. tested	AFB stain Positive	LJ-culture positive
20 - 29	38	1	1
30 - 39	135	9	9
40 - 49	255	13	18
50 - 59	322	17	30
Total	750	40	58

The study had found that the T.B. infection was observed with high percent in males and low percent in females (Table 3).

Table -3: Sex distribution of smear and culture-positive.

Sex	No. tested	AFB stain Positive	%	LJ- culture positive	%
Male	440	25		32	
Female	310	15		26	
Total	750	40		58	

***X`*=0.522 P=0.46**

The sensitivity, specificity, and negative predictive values (NPVs) of the sputum smear examination were calculated by using the sputum culture results as the "gold standard." (Table 4). The Sensitivity, Specificity, diagnostic accuracy, positive and negative predictive values of AFB stain were (68.9 %, 88.2 %, 73.3 %, 95.2 %, and 45.4 %) respectively and according to the following equations.

Table -4: Validity of AFB Stain for the diagnosis of TB was confirmed with results of L-J media.

Staining Methods	Culture result		Total	Sensitivity (%)	Specificity (%)	Predictive value (%)		Accuracy rate (%)
Ziehl-Neelsen	positive	negative				positive	negative	
positive	40 *T.P*	2 *F.P*	42	68.9 %	88.2 %	95.2 %	45.4 %	73.3 %
negative	18 *F.N*	15 *T.N*	33					
Total(%)	58	17	75					

* *T.P= True positive* ** *F.P = False positive*
*** *T.N= True negative* **** *F.N = False negative*

While the sensitivity, specificity, diagnostic accuracy, positive and negative predictive values of L.J. culture media were (95.2 %, 45.4 %,73.3 %, 68.9 %, 88.2 %) respectively.

Thus, culture technique remained the gold standard diagnostic Methods for tuberculosis. Culture Methods are highly sensitive and specific than microscopy for detection of bacilli, since approximately 10-100 mycobacteria per milliliter of sample is required for positive result while approximately 10000 bacilli /ml of sputum is required to be seen by microscopic examination (16).

But even when culture facilities are available, microbiological treatment is started on the basis of arbitrary clinical criteria and lack of response to other treatments. So to provide the accurate diagnosis of pulmonary T.B; the culture should always be requested concomitantly with A.F.B. smear where the culture facilities are available.

Culture requires at least a moderately well–equipped laboratory and necessarily lengthy time for its isolation and identification. So the cost and complexity associated with culture restricted its use only in major centers.

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