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Buffy Coat in Diagnosis of Pulmonary Tuberculosis

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ABSTRACT

***Background**: Besides charging the patients with outstanding costs, tuberculosis (TB) causes high mortality and morbidity in a country. We studied the sensitivity, specificity, positive and negative predictive values as well as the efficiency of buffy coat smear test in patients who were pulmonary TB suspects. This research was conducted at Massih Daneshvari Hospital, National Research Institute of Tuberculosis and Lung Disease (NRITLD).*

***Materials and Methods**: According to clinical and radiographic records of 50 pulmonary TB suspects, five ml of blood along with smear and culture of sputum were collected.*

The research method was a clinical trial (Diagnostic test type), and the technique was of observational-interview type.

Six buffy coat smears were obtained by Ficoll-Hypaque sedimentation method while the samples were stained by Ziehl-Neelsen stain.

***Results**: On sputum examination, 32 patients (64%) were B.K positive while 22 (44%) had positive sputum culture for Mycobacterium tuberculosis.*

Buffy coat was positive in 4 patients (8%). In comparison with sputum smear and culture, buffy coat had sensitivity of 12.5% and 13.6%, specificity of 100% and 96.4%, positive predictive value of 100% and 75%, negative predictive value of 39.4% and 58.7%, efficiency of 44% and 60% respectively.

***Conclusion**: In regard to the high specificity of buffy coat as compared to sputum smear (100%) and sputum culture (96.4%), it is possible to consider buffy coat as a method for screening tuberculosis patients that cannot expectorate sputum. Since buffy coat method has a high positive predictive value as compared to sputum smear (100%), it could replace other unavailable accurate methods like sputum culture and PCR and be used as a substitution for sputum smear.* **(Tanaffos 2003; 2(7): 41-45)**

***Key Words**: Buffy Coat Smear, Buffy Coat Culture, Tuberculosis, Diagnosis*

INTRODUCTION

TB is the cause of 3 million deaths in the world (1). This disease is a major health problem and issue in our country resulting in not only high costs but also increased mortality and morbidity rates. At present, besides clinical and radiological methods used for diagnosing TB patients, other laboratory techniques such as sputum smear with Ziehl-Neelsen stain, sputum culture in Lowenstein medium, and PCR are used to detect the genome of the organism

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(1,2). The diagnosis of TB and, hence, its treatment are usually based on clinical findings and radiological features. However, in this method, some of the patients are undiagnosed or misdiagnosed. Both of these conditions result in various problems and complications. The reason behind the above mentioned fact is the presence of some limitations including: the inability of the patient to expectorate sufficient sputum, patient's financial problems in referring to laboratories, high cost of PCR, and long period of evaluation resulting in inaccuracy of sputum culture reports.

Numerous studies have been performed regarding the use of buffy coat in diagnosis of pulmonary TB (3,4,5,6,7).

Since it is usually difficult to obtain sputum from some of TB suspect cases, more invasive methods such as bronchoscopy are needed, which results in treatment delay. Thus, it seems logical to discover a method for which sputum of TB suspects is not needed for their evaluation.

Considering the above fact, this study was conducted in order to compare the specificity, sensitivity, positive and negative predictive values, and efficiency of buffy coat with sputum culture and smear for the evaluation and diagnosis of pulmonary TB.

MATERIALS AND METHODS

Between the year 2000 and 2001, a total of 50 (27 female, 23 male) pulmonary TB suspects were selected from those referring to “Massih Daneshvari Hospital” (NRITLD). The research method was a clinical trial (diagnostic test), and the technique was observational-interview type.

After obtaining the necessary permits all of the selected pulmonary TB suspects who had referred to pulmonary clinic of “Massih Daneshvari Hospital” were evaluated. Initially a history was obtained. If the patient's history revealed clinical criteria compatible with TB (fever, sweating, cough with sputum, weight loss or hemoptysis) and the infectious specialist judgment was that of "TB suspected case", a chest x-ray was obtained. If the radiologist believed that the chest x-ray had manifestations consistent with TB (bilateral lung involvement, involvement of several lobes of lung especially upper lobes, presence of cavitation, lymphadenopathy and or pleurisy), the patient was considered as a pulmonary TB suspect. Then, in addition to sputum smear and culture, 5cc blood was obtained and sent for buffy coat smear (by Ficoll Hypaque Sedimentation method) to laboratory. The patients' data were recorded in form No. 1.

In this study, 5cc of heparinized blood sample was mixed with 5cc of RPMI solution. Two cc of Ficoll solution was added to a graduated plastic tube. Then, the diluted blood was slowly added to the plastic tube containing Ficoll solution, in such away that the Ficoll solution remained at the bottom. The sample was then centrifuged with 4000 rpm for 30 minute. After centrifugation, red blood cells (RBC), buffy coat, and serum were present in the lower, middle, and upper part of the tube respectively. The upper serum layer was slowly thrown away and the buffy coat was then transferred to another plastic tube. In order to eliminate Ficoll solution, 5cc of phosphate buffer solution (PBS) was added to the tube containing buffy coat and then mixed carefully. This mixture was subsequently centrifuged with 3000 rpm for 15 minute. The upper solution was discarded, and a slide was prepared from the sediment. The slide was air fixed, fixed with methanol, stained with Ziehl-Nelseen method, and examined under the light microscope.

The buffy coat smear was examined for M.tuberculosis by a microbiologist, and information was noted down in form No.2.

The individual that examined the buffy coat smear was not aware of sputum culture, sputum smear and the radiologists CXR report noted in form No.1.

The data were then analyzed for sensitivity, specificity, positive and negative predictive values, and efficiency.

RESULTS

In this research 50 patients (27 female, 23 male) having a mean age of 49.5±19.7 yrs. were evaluated.

The sputum smear was positive for AFB in 32 patients (64%) while it was negative in 18 (36%). Also a positive sputum culture for M.tuberculosis was found in 22 (44%), and a negative sputum culture was seen in 28 patients (56%).

Buffy coat examination was positive in 4(8%) patients and was negative in 46 (92%) patients.

Sensitivity, specificity, positive and negative predictive values, as well as efficiency of buffy coat in comparison with sputum smear and culture are shown in Tables 1 and 2 respectively.

Table 1. Distribution of patients suspected of having pulmonary tuberculosis based on results of sputum smear and buffy coat examination (2000-01)

Buffy coat smear	No. of Patients (positive sputum smear)	No. of Patients (negative sputum smear)
Positive	4	0
Negative	28	18

Sensitivity: 12.5%; specificity= 100%; positive predictive value = 100%; negative predictive value = 39.4%; efficiency = 44%

Table 2. Distribution of patients suspected of having pulmonary tuberculosis based on results of sputum culture and buffy coat examination (2000-2001)

Buffy coat smear	No. of patients (positive sputum culture)	No. of patients (negative sputum culture)
Positive	3	1
Negative	19	27

Sensitivity = 13.6%; specificity 96.4%; positive predictive value = 75%; negative predictive value = 58.7%; efficiency = 60%

DISCUSSION

Only 4 patients (8%) had positive buffy coat (with Ziehl Neelsen Staining) which is very much less than the results of Sen's et al. study (3). In the study conducted by Sen et al., 55% of buffy coat smears were positive. The reason behind this could be explained by the fact that all of the studied patients in Sen's research suffered from "positive smear pulmonary TB". Also, our finding is in contrast to that of Richter study (4). Richter found positive buffy coat culture in 13% of the patients under study, while all of buffy coat smears were negative. Richter's study was performed on "HIV positive pulmonary TB patients", which could explain the above-mentioned difference.

As seen in our research, the sensitivity of buffy coat in diagnosing pulmonary TB is less than sputum smear (12.5%) while the specificity of buffy coat as compared to sputum smear is similar i.e. 100%. Also, the positive predictive value of buffy coat in comparison with sputum smear is 100%. In other words, if buffy coat smear is positive in a pulmonary TB suspect, the patient's sputum smear is 100% positive. The negative predictive value of buffy coat smear is less than that of sputum smear (39.4%). This means that in a pulmonary TB suspect, if the buffy coat smear is negative, there is a 40% chance of having negative sputum smear.

The above points were not investigated by Sen (3), Richter (4), Ruf (5), Eng RH (6), and Damsker (7). The efficiency of buffy coat is less than that of sputum smear (44%). It seems that for diagnosing pulmonary TB suspects, buffy coat smear is less frequently used as compared to sputum culture. Its specificity in ruling out the disease in normal individuals is 96.4%. The positive predictive value of buffy coat in comparison with sputum culture is 75%. This means that if buffy coat is positive in a patient, there is 75% chance of confirmation of the

disease by sputum culture. The negative predictive value of buffy coat as compared to sputum culture is 58.7%; that is, if the buffy coat of a patient is negative, there is 59% possibility that sputum culture does not confirm the disease. Previously conducted studies had not reached this conclusion. (3,4,5,6,7).

With regard to the above mentioned facts and the high specificity of buffy coat smear in comparison with sputum smear (100%) and sputum culture (96.4%), it could be possible to substitute buffy coat smear for the two aforementioned procedures in order to screen patients and find those that do not have pulmonary TB. In addition, high positive predictive value of buffy coat smear as compared to sputum (100%) gives the chance to use buffy coat smear instead of sputum smear in the absence of more accurate diagnostic procedures such as PCR and sputum culture. However, because of few studies conducted in this field, we strongly recommend more comprehensive assessments along with more number of sample examination for more accurate evaluations.

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