

Comparison between Fecal Occult Blood (FOB) Device with Ziehl-Neelson (Z.N) Stain and Polymerase Chain Reaction (PCR) Techniques in the Diagnosis of Early Pulmonary Tuberculosis (PTB)

Yousif M Alobaid¹, Amani S Elsheikh², Mosab Nouraldein Mohammed Hamad^{3*}, Mogahid M. El Hassan⁴

¹Department of Microbiology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Sudan

²Soba Teaching Hospital, Registrar of Pediatrics, Khartoum, Sudan

³Phylum of Medical Parasitology, Medical Laboratory Sciences Department, Faculty of Health Science, Elsheikh Abdallah Elbadri University, Berber, Sudan

⁴Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taibah University, KSA

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*Corresponding author: Mosab Nouraldein Mohammed Hamad

Abstract

The aim of this study was to evaluate the sensitivity of Fecal Occult Blood (FOB) strips in the diagnosis of Pulmonary Tuberculosis (PTB) isolated from sputum of tubercles patient. The study was carried out in Omdurman Teaching Hospital-Abu Anga-Tuberculosis ward. From 27th of August to 25th of September 2007. A total of 67 sputum samples collected from the suspected people. The samples examined by FOB test and Z.N techniques. Ten out of the enrolled samples, which showed positive FOB and negative Z.N techniques selected confirmed by using PCR techniques. The result showed that there were 12 (17.9%) of cases were Z.N (+ve), while 55 (82.1%) of cases were Z.N (-ve). It was also showed that there were 53(79.1%) of cases were FOB test (+ve), while 14(20.9%) of cases were FOB test (-ve). According to PCR techniques, after performed 10 samples that showed Z.N (-ve) and FOB test (+ve), it was noted that there were 4(40%) of cases were PCR (+ve), while 6(60%) of cases were PCR (-ve).

Keywords: Sensitivity, FOB Strips, Pulmonary Tuberculosis, PCR, Sudan.

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INTRODUCTION

The World Health Organization (WHO) recommends the household tuberculosis (TB) contact investigation in low-income countries, but most contacts do not complete a full clinical and laboratory evaluation [1].

Recently, there has been an increase in cases of tuberculosis that may give to the increase in tuberculosis infection in, an increase in HIV infection, increase number of homeless people and the appearance of drug-resistance strains of tuberculosis [2].

The symptoms of people who have a pulmonary TB are, hemoptysis (coughing up blood), fatigue, weight loss, fever and night sweats, phlegm-producing cough, wheezing, chest pain, and breathing difficult [3].

The diagnosis of pulmonary tubercle patient by many ways, the tests may include: Microscopic examination, these by using Zeihl-Neelsen (Z.N)

Staining techniques for sputum or other smear suspected, Culture techniques, these for culturing Sputum. Tuberculin skin test, these used to check the patients if exposed to TB or not. Serological tests, as immuno-chromatographic strips (ICTs), DNA detection, these by using Polymerase Chain Reaction (PCR). Chest X-rays, these conduct by pictorial of the lungs. Bronchoscopy, interferon (IFN)-Gamma blood test, and Rarely, biopsy of the affected tissues (lungs and lymph nodes) [2].

Rationale

New strategies required to diagnose early cases of pulmonary tuberculosis to avoid the progress of a disease as well as Zeihl-Neelsen techniques with a sensitivity of 70%, but a 30% considered as misdiagnosed. So detection of occult blood in sputum of pulmonary tuberculosis patients may offer a suitable test for early diagnosis.

MATERIALS AND METHODS

A cross-sectional study designed to target a 67 suspected people who present with signs and symptoms

of pulmonary tuberculosis and referred to the TB laboratory aiming to detect Mycobacterium tuberculosis bacilli in their sputum specimens. Data collected using standard questionnaire.

Methods of Zeihl-Neelsen Techniques

Thin smears prepared, air-dried, then it covered by carbol-fuchsin (appendix 1-1) for 5 min and heated with flame without boiling, then the smears washed by tap water and then decolorized by 20% sulphoric acid (appendix 1-2) for 5 min and washed by tap water and then methylene blue (appendix 1-3) was poured for 3 seconds then washed by tap water and finally examined microscopically under oil immersion for AFB.

Occult Blood Test

This is conduct by using Umedic One Step Cassette Style Fecal Occult Blood (FOB) test (appendix 2-2). As follows; the patients were instructed to get early morning sputum in a clean, wide leak-proof container. 3-5 loop full (appendix 2-1) of samples taken and emulsified into 2ml of buffer solution (appendix 2-1) Then it shaken well and transferred 3-4 drops from the suspension to the well of the device. Then the sample was left 5-10 min and matched the test band with control band (Fig 1 and 2).

Polymerase Chain Reaction (PCR)

There are many types of PCR, each of them has steps including DNA extraction (appendix 3-1) and then PCR machine (appendix 3-4).

Method of PCR

The solution (DNGTM) (appendix 3-1) was at 37 ° C in water bath for 30 seconds and safety shake. Then the Eppendorf tube (1.5) labelled by the No. of sample, then it homogenized by using vortex for several seconds and shaken well. 100µl of sample taken into the labelled Eppendorf tube (1.5). Then 400µl of DNG solution added to each sample, and shaken well and centrifuged at 12.000 rpm for 10min. Then 300µl of Isopropanol added and shaken gently and centrifuged. The supernatant discarded. Then 1ml of 75% ethanol (appendix 3-8) added to each sample and gently shaken and centrifuged at 12.000 rpm for 5min (as washed for 2-3times), then the samples blotted by using tissue paper. The water bath (65° c) was used to dry the pellet of DNA for 5 min then 50µl of D.W added, then vortex

and centrifugation for 30 seconds done. The pellet of sample put in the PCR machine (Fig-3) and left up to complete the PCR cycles (appendix3-4). Then master mix (appendix3-3), (1.5% w/v) agarose (gel), 10% TBE (appendix 3-6), and 10% Ethidium bromide fluorescent dye (appendix 3-7) ready. The 10% TBE poured to the gel and 10% of fluorescent dye added. Then 3µl of methylene blue (appendix 1-3) dye taken and put into a well of a micro-titrate plate, then 7µl of the sample taken and mixed well with dye and transferred to the gel cell up to 30 min at 50 volts. The result read by transferred it to the Gel documentation system by using UV light as in (Fig-4) Photo get out by computer as in (Fig-3).

RESULTS

This survey performed in Omdurman Teaching Hospital-Abu Anga – Unit of Tuberculosis. 67 suspected individuals included in the study and the results showed that: According to Z.N techniques, it noted that there were 12 (17.9%) of cases were Z.N (+ve), while 55 (82.1%) of cases were Z.N (-ve) as in table 1. According to FOB test, it was noted that there were 53 (79.1%) of cases were FOB test (+ve) (Fig-2), while 14(20.9%) of cases were FOB test (-ve) (Fig-1) as in Table-2. According to PCR techniques, after performed 10 samples that showed Z.N (-ve) and FOB test (+ve), it was noted that there were 4(40%) of cases were PCR (+ve), while 6(60%) of cases were PCR (-ve) as in Table-3.

Table-1: Shows the result of Z.N techniques of 67 patients

The result	No. of patients	percentage
Positive	12	17.9%
Negative	55	82.1%

Table-2: Shows the result of FOB test of 67 patients

The result	No. of patients	percentage
Positive	53	79.1%
negative	14	20.9%

Table-3: Shows the result of PCR techniques of ten patients

The result	No. of patients	percentage
Positive	4	40%
negative	6	60%



Fig-1: Negative control band (C) only

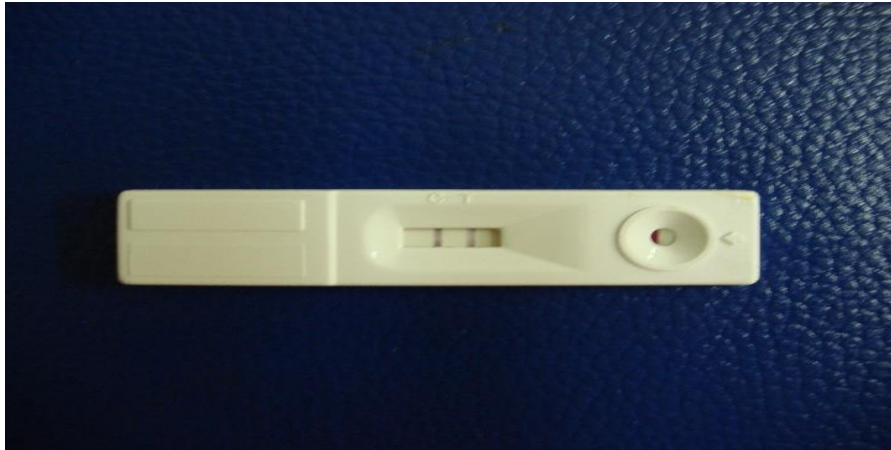


Fig-2: Positive control band (C) and test band (T) both



Fig-3: Shows putting the samples into DNA Thermo cycler



Fig-4: Shows the UV light cabinet

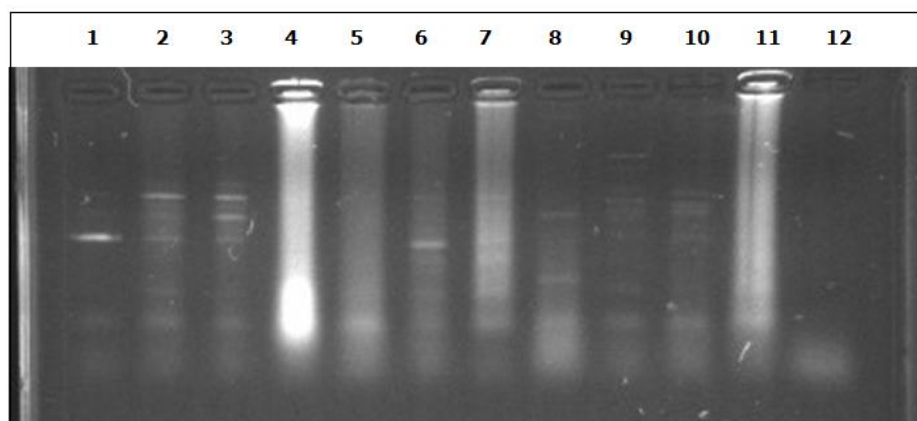


Fig-5: Polymerase Chain Reaction run on a 1.5 % w/v agarose gel, 50 Volts, stained with Ethidium Bromide. DNA size marker is a commercial 173 bp. The position of the wells and direction of DNA migration is noted. The numbers of samples in an image indicates the serial number of patients in a Hospital. The pictorial show samples No. 2 , 3 , 6 & 7 are positive; 1 is (positive control), 5 , 8 ,9 , & 10 are negative ; 12 is (Negative control) but 4 , 11 aren't consider due to smearing.

DISCUSSION

In this study evaluation of FOB strips conducted by matched the result of FOB test strips with Z.N and polymerase chain reaction (PCR) techniques. Also there is no so much published data about the use FOB strips in detection of occult blood in TB patients, but such technique have been widely used in similar occasions in the patients with ulcer, urinary tract infection, as mentioned by [4]. Chi-square used, it revealed that, there was association between the uses of FOB strip and the established methods for detection of the pulmonary tuberculosis patients.

The FOB strips were matched to Z.N techniques and it shown that 12(18%) of cases had the disease while truly had a disease and 41(61%) of cases were disease by using FOB test while did not have a disease by using Z.N technique, and 14(21%) of cases were freely of a disease by both methods.

10 specimens out of 41 cases performed by PCR, it shown that 4(40%) of cases truly had a disease while 6(60%) of cases were free of the disease. When it was considered in every 10 cases { Z.N (-ve), FOB (+ve)} there were 4 cases positive for the disease, so in 41 cases there were 16 were had a disease and by addition of these 16 cases to whom a diseased, it was became 28(42%) of cases were truly positive while by subtraction these 16 cases from 41`cases were became 25(37%) of cases were positive by FOB test and

negative by Z.N techniques, but the number of people who did not have the disease became constant 14(21%).

CONCLUSION AND RECOMMENDATIONS

From the obtaining results and after comparing the results of FOB with the results of PCR. Obviously, the FOB strips used as screening test for the pulmonary tuberculosis patients. If the test is positive need confirm by a well-established methods like PCR techniques. Further studies required with large sample size and with several diagnostic methods.

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