Implementation of a Rapid and Low-cost Test of Pulmonary Tuberculosis among Suspected Individuals

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Abstract: In this project, we aimed to develop a new rapid and low-cost screening test to detect pulmonary tuberculosis among suspected subjects. A total of one hundred and seventy-four (n=174) individuals, who attended Abu-Anga Hospital for chest diseases and Al-Shaab Teaching Hospital (Sudan) were included in this project. ZN stain was performed seeking for the presence of acid-fast bacilli in their sputum samples. Occult blood (OB) in all sputum samples was also investigated with the aid of special commercial strips. Furthermore, we selected eighteen sputum specimens which appeared positive with (OB) and negative with ZN technique to be tested for the presence of a 123 bp DNA band by amplifying *IS 6110* as a conservative region in *Mycobacterium tuberculosis* complex, using PCR technique. Our results proved that 38/174 samples (21.8%) were ZN positive whereas 136/174 specimens (78.2%) were ZN negative. For OB, 139/174 samples (79.9%) were positive whereas 35/174 (20.1%) appeared as negative. Shifting to PCR results 10/18 sputum specimens (55.6%) were positive while the remaining 8/18 (44.4%) appeared as negative. In conclusion and putting into consideration some exclusion criteria with minor modifications, the use of OB strips will be a suitable screening technique to investigate pulmonary tuberculosis especially in remote areas where specialized well-equipped laboratory are scarce.
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1. Introduction

Human tuberculosis (TB) is chronic diseases which may be caused by one of five closely related species, known as *Mycobacterium tuberculosis* complex. The diseases can also be caused by mycobacteria other than tuberculosis [1, 2, 3]. TB is primarily a disease of the lungs. It may affect the healthy individuals as well as the immunocompromised particularly AIDS patients [4].

TB is spread worldwide, with a special focus on Asia and Africa. About 2 billion people, a third of the world's population, are infected [5].

TB is a leading cause of morbidity and mortality worldwide, and it has been for centuries, one of the most prevalent infectious diseases [6]. Recently WHO estimated that the situation in poor developing countries, namely in sub-Saharan Africa, is deteriorating very rapidly. The reports suggested that more than 8 million new cases and around 2-3 million deaths annually. Hence TB is still a major health problem leading to death, and its eradication will be so difficult as long as malnutrition and poverty covered a huge part of the Earth [7, 8].

As in the other part of Africa, epidemicity of TB in Sudan was affected by many factors including malnutrition, poverty, and increasing number of displaced peoples. Insufficiency of health and laboratory requirements may also play a role in the epidemic of TB [9].

Diagnosis of pulmonary tuberculosis (TB) in developing countries like Sudan is challenged by lacking well equipped laboratories as well as experts personnel [10]. For more than one hundred years, tuberculin skin test (TST) was the golden diagnostic test for *Mycobacterium tuberculosis* infection. It was introduced previously by the German scientist Robert Koch In 1882. He prepared it by boiling the mycobacterial culture and introducing it into patients as a treatment for tuberculosis infections. The outcome of this experiment was a discovery of a definitive means to identify *M. tuberculosis* infection which is TST [11].

The diagnostic scheme of this disease is based on the isolation and characterization of the causative microorganisms in an appropriate laboratory setting. The conventional techniques used in the diagnosis of TB are microscopy using different types of stains; this method gives rapid results with the minimal level of specificity and sensitivity. Another reliable technique is culturing of the causative organisms on special media, time needed to obtain a visible growth with final identification results in this method is 4-8 weeks.

Also, DNA probes techniques are used to identify MTB complex, and it takes several hours but reflects high sensitivity and specificity [12]. Thus, establishing and improving new, ultra rapid diagnostic tests is highly recommended.

2. Materials and Methods

Study type, population, and sampling

A cross-sectional study was designed to target a population of 174 patients 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 were collected using standard questionnaire.

Occult Blood Test

One step right test for occult blood was used (from U media, Karmannsstrasse 57. 41061 Moenchengladbach, Germany) as follows; using a microbiological loop, 3-5 loopful of sputum sample was transferred to the sample collection chamber. We mixed it with 2 ml of solution A, then 2 to 3 drops of the mixture was moved to the special sample pad provided with the test, and finally the result was registered 5-10 minutes after incubation.

3. Results

This project aimed to develop and establish a new ultra-rapid and reliable screening technique for the diagnosis of PTB. Sputum samples were obtained from 174 patients; all samples were examined for the presence of AFB by ZN stain and further for the presence of OB.

The results showed that: thirty-eight (21.8%) of the study subjects were ZN +ve and 136 (78.2%) were ZN -ve, whereas 139 of the enrolled subjects (79.9%) were OB positive and 35/174 (20.1%) were negative for OB (Table 1 and Figure 1). The selected eighteen sputum specimens, which were ZN -negative and OB positive, were tested for the presence of a band of 123 bp within *IS6110* using PCR. The results proved that 10/18 (55.6 %) were positive for target band whereas the remaining samples 8/18(44.4) were negative (Table 1 and Figure 2).

Table 1. Results of ZN and OB tests performed on 174 patients with symptoms of tuberculosis in different hospitals and medical centers in Khartoum State, Sudan

Total	Sputum Blood		Occult
36 138	Negative 4 31	Positive 32 107	ZN Positive ZN Negative
174	35	139	Total

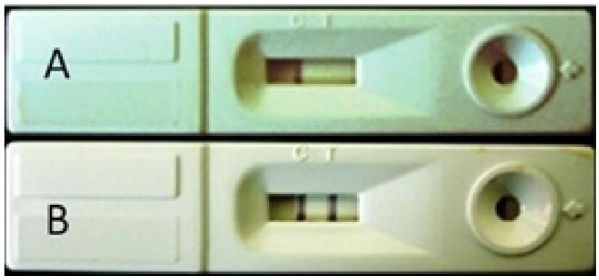

Figure 1. Occult blood strips in the diagnosis of pulmonary tuberculosis: strip (A) shows negative result; strip (B) shows the positive result.

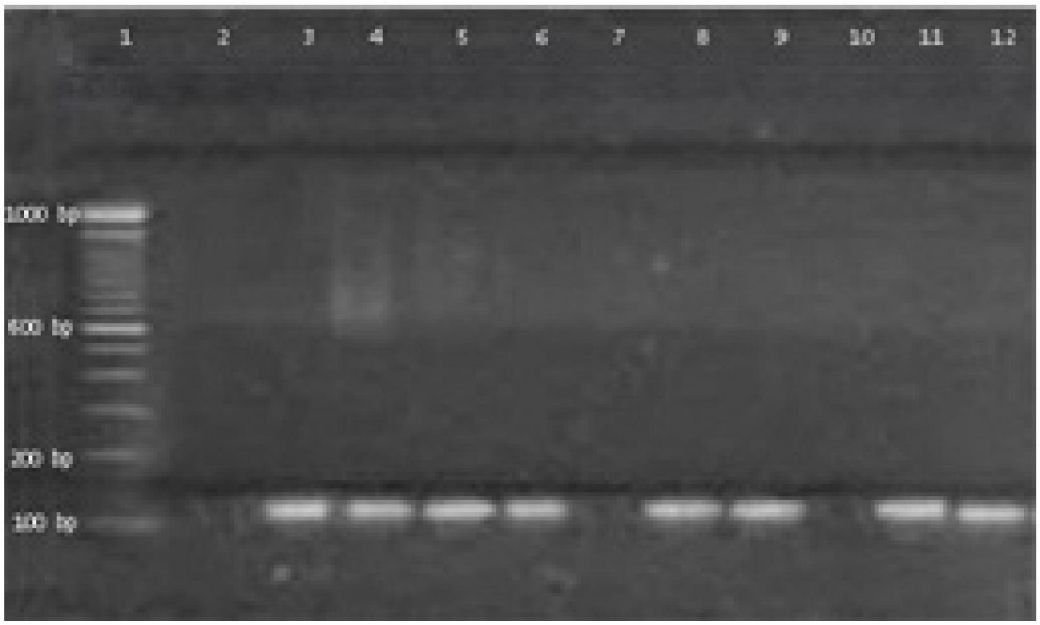

Figure 2. An ethidium-bromide stained gel containing amplicons of *IS6110* produced by PCR; Lane 1 is MW marker; Lane 2 is control -ve, lanes 3, 4, 5, 6, 7,8, 9, 10, 11 and 12 show different patients' samples 32.

4. Discussion

Whilst TB is responsible for around 2 million deaths and 8 million new cases every year and because the growth of the causative agents may take a long time in isolation, identification and antimicrobial sensitivity testing (DST), searching for ultra-rapid and reliable screening test is strongly recommended. Previously many serological and molecular techniques have been introduced for the detection of mycobacteria. These innovated techniques can reduce the required time from weeks to days [13]. In this project we aimed to study the use OB strips and evaluate its results with ZN stain as well as PCR from direct sputum samples. Among the enrolled patients (n=174), smears were positive only in 38/174 (21.8%), this may attributed to the fact that ZN stain is usually needed more than 500 bacilli/ml to work properly [14]. This statement was supported by (Kavita et al., 2006) [15] who reported that the sensitivity of ZN was < 50%. Similar findings were also reported by Elhassan et al., 2016 [10]. In contrast, OB was reported as positive in 139/174 (79.9%). Obviously this may reflect the high sensitivity of OB to diagnose one important sign of tuberculosis which is the presence of blood within sputum samples.

Ten of the eighteen selected samples (55.6 %) which were ZN -Ve and OB +Ve appeared as positive for *IS6110* with the band of 123 bp proved that PCR is more reliable than ZN stain. Similarly, Aroma, (2007) and Elhassan et al., 2016 [10,16] listed that PCR showed the highest sensitivity compared to other diagnostic tests. Eight OB-positive samples (44.4%) were *IS6110* negative. This may explain the presence of PCR inhibitors in these eight sputum samples, similar statement were listed before by Maher, (1996) [17], or this failure of PCR may be true negative results and the presence of occult blood in those patients samples may be due to other diseases capable of inducing bleeding within the buccal cavities, thus, false positive results for OB strips may be reported.

In conclusion, the results of OB strips are encouraging thus, can be used for primary screening of highly suspected clinical cases especially in epidemic areas as a quick and easy to perform test. However, we still need to pay attention for the chance of false positive results when using OB strips. Finally, we do recommend PCR as a reference method in the diagnosis of pulmonary tuberculosis among suspected cases.

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