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Research Article

PCR IS--1081 Gene-based Detection of Mycobacterium Tuberculosis Complex in lymphocytic Pleural Effusion Samples of Sudanese Patients

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Abstract

Introduction: Tuberculosis (TB) has the potential to be a serious health problem in Sudan. Early laboratory confirmation of TB can lead to early initiation of treatment and increased opportunities to interrupt transmission. Nucleic acid amplification tests are widely used for detection and species differentiation of Mycobacteria in clinical specimens. This study aimed to detect specific members of Mycobacterium tuberculosis complex in lymphocytic pleural effusion. 88 pleural effusions were cytologically examined, of which 52 were diagnosed as showing chronic inflammatory reactions. The pleural effusions were from Sudanese patients clinically suspected to have pulmonary tuberculosis. DNA was isolated using DNA extraction kits from Vivants (Malaysia). PCR Amplification of the target sequence (IS-1081 gene, 344 bp) was carried out and then analyzed using agarose gel electrophoresis. Out of the 52 studied samples of lymphocytic pleural effusions, 22 (50%) of the DNA positive samples were positive for IS-1081 gene. Molecular assays of Mycobacterium tuberculosis complex can provide rapid diagnosis of the tuberculosis which is one of the major causes of pleural effusions in general and lymphocytic effusions in specific in Sudan.

Keywords

Tuberculosis, TB, Mycobacterium Tuberculosis Complex, PCR, IS-1081 Gene

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1. Introduction

Tuberculosis still remains an important cause of morbidity and mortality worldwide. Recent estimates showed that 10.8 million had tuberculosis (TB) in 2023 [1] and 2-3 million die annually [2]. African region has the highest estimated incidence which is 356 per 100,000 populations per year (Gopi, *et al* 2007).

TB is considered one of the most frequent causes of pleural effusions worldwide, even in developing countries [3-5]. The frequency of pleural involvement in TB has been variably reported: 3%-5% in United States [6] and exceeding 20% in Spain [7] and Nigeria [8].

Tuberculosis is difficult to diagnose in pleural effusions, which is usually a secondary immunologic response to the rupture of the bacilli tubercles this is due to the presence of only a few organisms reaching the pleural fluid in the early stage of tuberculosis infection [9, 10]. Traditional methods used for identification of tuberculosis have limitations; acid-fast staining is neither sensitive nor specific and culture is time consuming and of low sensitivity [11]. Nearly 40% of patients with active tuberculosis remain undiagnosed [12]. In Sudan, the case detection rate was estimated to be 36.5% in 2007, which is far below the target of 70% [13]. The rapid detection of *Mycobacterium tuberculosis* is of importance to effectively reduce transmission of infection in patients [14].

Polymerase chain reaction (PCR) yields quick results by amplifying specific DNA sequences, even if only a single copy of a given DNA sequence is available [11]. The sensitivity of detection of mycobacteria could be improved by PCR which can detect as few as 10 organisms. Advantages of PCR include rapid diagnosis, improved specificity and sensitivity, and no requirement of intact immunity [15] Various PCR-assays have been devised for identification of tuberculosis in clinical specimens [11]. The presence of multiple copies of IS-1081 gene in all strains belonging to *Mycobacterium tuberculosis* complex make these strains easy to detect in tuberculosis patients [16].

Mycobacterium tuberculosis complex, including *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium africanum* and *Mycobacterium canetti* have shown a great similarity of DNA sequence among the members of the *Micobacterium tuberculosis* complex [17].

The aim of this study is to estimate the magnitude of *Mycobacterium tuberculosis* complex in lymphocytic pleural effusion using single PCR IS-1081.

2. Methodology

Retrospective study of eighty-eight aspirations of pleural fluid were examined to determine the cytological profile of preserved pleural effusions of the male and female Sudanese patients submitted to Khartoum Teaching Hospital and Dr. Elmobarak laboratory during the period of 2008-2011. According to the method described by [18] conventional Papanicolaou and May-Grünwald-Giemsa stains were used.

Molecular examination of the samples that showed lymphocytic pleural effusion was carried out using conventional PCR technique to detect *Mycobacterium tuberculosis* complex IS-1080 gene in Sudanese patients with lymphocytic pleural effusion.

DNA extraction was performed, as described by the manufacture, using Vivantis kits, Malaysia. DNA was stored at -20 ℃. The extracted DNA was detected by electrophoresis before amplification. Samples with good DNA were the only used ones.

As described previously by [16], a pair of primers that enclosed a 344-bp fragment of the IS-1081 gene *Mycobacterium tuberculosis* complex was used in PCR assay.

Table 1. *Primer Sequences Used for PCR Amplification of the IS1081 Gene of the Mycobacterium Tuberculosis Complex.*

Type	Primer	Sequence
TB IS-1081	Forward primer	5′- CGAATCAGTTGTTGCCCAAT-3′
	Reverse primer	5′-GTTCTTCGGTGCTGGTCAGT-3′

Samples were amplified by PCR in a 25 µL reaction mixtures containing: 1 µL dNTP (10 mM), 1 µL of each primer (20 pM), 0.25 µL (500 U) Taq polymerase, 1.5 µL $MgCl_2$ (25 mM), 2.5 µL of 10X PCR buffer and 2 µL of DNA template [19].

The amplification parameters included an initial denaturation at 95 ℃ for 5 min followed by 40 cycles each at 95 ℃ for 30 sec, annealing at 56 ℃ for 30 sec and extension at 72 ℃ for 45 sec. The extension step in the 35^{th} cycle was held for 5 min before the samples were detected. DNA was detected by electrophoresis on gels and stained with ethidium bromide [16]. Data were recorded and then were analyzed using chi-square test.

3. Results

Cytological examinations of the pleural effusions among Sudanese patients collected in four years revealed 63 (71.6%) positive smears and 25 (28.4%) negative smears. Among the positive smears 52 cases (82.5%) showed lymphocytic inflammation, of these 31 cases were from males and 21 cases were from females.

Out of the 52 pleural effusion samples that had exhibited lymphocytic effusion, 44 yielded good quality DNA. PCR analysis of these 44 DNA samples revealed that 22 (50%) samples were positive for *Mycobacterium tuberculosis*

IS-1081 gene while the remaining 22 (50%) samples were negative.

4. Discussion

Tuberculosis (TB) remains an important public health problem worldwide [19, 14]. Although it is curable and preventable [20], it is one of the biggest killers among the infectious diseases [21]. Globally, in 2019, among HIV-negative individuals, there were 1 18 million (95% uncertainty interval 1.08-1.29) deaths due to tuberculosis and 8 50 million (7.45-9.73) incident cases of tuberculosis [22]. In 2023, it is considered as the leading infectious disease killer worldwide [19].

In this study, 44 DNA samples from pleural effusions of Sudanese patients were tested for presence of IS-1081 gene using single PCR assay, 22 (50%) of the samples were found to be positive. Tuberculous effusions constituted at least 42% of the total lymphocytic effusions. This finding is consistent with the known predominance of lymphocytic exudates in tuberculous pleural effusion. The higher frequency of lymphocytic inflammation in males compared to females may reflect previously reported trends in tuberculosis prevalence, which has been shown to affect men more commonly than women in endemic regions. [22] The percentage of tuberculosis in Sudanese patients' pleural effusions is almost similar to that recorded by [23, 4]. In Egypt, a prospective study found that 64% of patients presenting with exudative pleural effusion had tuberculous etiology [24]. A study in Ethiopia reported that 64.8% of pleural effusion cases had lymphocyte-predominant fluid, which was statistically associated with tuberculosis. These regional data align with the Sudanese findings, reinforce the need for targeted TB diagnostic approaches in pleural effusions across similar epidemiological conditions. Sudan has one of the highest burdens of the disease in the eastern Mediterranean region [20] accounts for 8%-15% of that burden [25, 13]. The lower percentage revealed in this study compared to regional countries may be due to the place from which the patients originate. Still the results revealed demonstrated that tuberculosis is one of the major causes of pleural effusions.

PCR is a highly effective and rapid method for diagnosis of tuberculosis pleural effusion using different genomic DNA sequences and extraction methods [16]. Molecular assays of *Mycobacterium tuberculosis* complex can provide rapid diagnosis of the disease. The percentage of tuberculous pleural effusion in this study (50%) indicates that other causes of pleural effusion also exist among the studied population. These results suggest investigation using different types of sample types such as lymph node aspiration, sputum, pleural biopsy and fecal samples to detect the most reliable sample type for TB diagnosis using PCR.

The IS-1081 gene has been described as a relatively sensitive and specific marker for detecting *Mycobacterium tuberculosis* complex. However, the detection rate in this study may reflects a limitation of using PCR as a stand-alone diagnostic tool. Thus, PCR should be considered complementary to cytology, clinical findings, and other microbiological methods such as culture, which remains the gold standard despite its longer turnaround time.

5. Conclusion

Molecular assays targeting the *Mycobacterium tuberculosis* complex enable rapid diagnosis of tuberculosis, a leading cause of pleural effusions in general and particularly of lymphocytic pleural effusions in Sudan.

6. Recommendation

It is recommended that PCR targeting the IS-1081 gene be incorporated into routine tuberculosis diagnostics for lymphocytic pleural effusions in Sudanese patients, as it identified TB in 50% of cases, demonstrating its effectiveness as a rapid diagnostic tool for this high-risk population.

Abbreviations

TB	Tuberculosis
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
IS1081	Insertion Sequence 1081
bp	Base Pairs
dNTP	Deoxynucleotide Triphosphate
HIV	Human Immunodeficiency Virus

Conflicts of Interest

The authors declare no conflicts of interest.

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