Original Article

Prevalence of suspected tuberculosis in the Kingdom of Saudi Arabia according to conventional and molecular methods

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ABSTRACT

Background: Although the prevalence of suspected tuberculosis (TB) in the Kingdom of Saudi Arabia remains high, there has been a modest decrease in recent years. In this multi-center cross-sectional study, the prevalence of TB was determined by various techniques with the aim of identifying differences and indicating where there is uniformity in findings. **Materials and Methods:** A total of 3404 samples were collected from Saudi TB patients from different regions in Saudi Arabia: Riyadh, Dammam, Jeddah, Madinah, Hail, Qassim, Jazan, and Taif. Different techniques including Ziehl–Neelsen (ZN), *Mycobacteria* growth indicator tube (MGiT), Lowenstein–Jensen (LJ), and polymerase chain reaction (PCR) were used to screen for the presence of *Mycobacterium tuberculosis* (MTB). **Results:** ZN stain showed that Riyadh and Dammam had the highest prevalence of MTB with 22% and 21%, respectively, while prevalence was lowest in Jazan and Hail with an incidence of 2% and 3%, respectively. MGiT culture showed that Riyadh, Dammam, and Jeddah had the highest prevalence with a rate of 26%, 22%, and 22%, respectively. LJ culture showed the highest prevalence in Riyadh and Dammam with 22% and 21%, respectively. Of all the techniques, the highest detection rate was by PCR which was 10.46% while ZN stain technique was 6.64%, for MGiT culture it was 8.34%, and for LJ culture it was 7.7%. **Conclusion:** This study is the first in which different methods have been used for detection in the various regions of Saudi Arabia. Collected data are important not only for patients and physicians but for future epidemiological studies to monitor the spread of MTB infection in Saudi Arabia.

Key words: Methods, Saudi Arabia, screening, tuberculosis

INTRODUCTION

Tuberculosis (TB) remains a major global public health problem. The World Health Organization estimates that 8 million new cases and 3 million deaths are directly attributed to the disease each year.[1] It is one of the leading causes of death in the world from a single infectious agent. The disease affects 2 billion people which is equal to one-third of the entire world population. Approximately 9 million people contract TB globally in a year and 2-3 million people die every year.[2] The increase in the incidence is mostly in Africa and Asia, where there is the highest prevalence of co-infection with HIV and *Mycobacterium tuberculosis* (MTB).[3]

In the Kingdom of Saudi Arabia (KSA), the latest prevalence rates of TB range from 8.5% in the central region (Riyadh) to as high as 23.1% in Hail for locals, and as high as 38% for non-Saudis in the Makkah region.[4] Fortunately, improvements in healthcare have led to a modest decrease in the incidence, despite the alarming prevalence levels,[5] which is probably the result of events in which there are mass gatherings such as the Hajj.[6]

In this cross-sectional study, the prevalence of TB in KSA is revisited, using different methodological approaches with the aim of finding out regional variations in incidence, as well as the differences in the rates revealed by the various methods.

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MATERIALS AND METHODS

In this multi-center, nationwide cross-sectional study, a total of 3404 Saudi subjects from different regions in KSA were included. These were random outpatients who came to the designated testing centers all over KSA for screening and diagnosis of TB. The work of the centers and hospitals was the collaborative effort of the Ministry of Health and the TB Laboratory based in King Saud Medical Complex in Riyadh, which is the reference laboratory for all the other centers included in the study. Convenient sampling was employed over a 3-year period (2008-2010). All subjects were instructed on how to collect sputum specimens on 3 consecutive days, and were provided with materials (kits) for the collection and transportation of the specimens. Ethical approval was obtained from the Ministry of Health, which also granted access to the different tertiary hospitals in order to retrieve biological samples for analysis. All samples were analyzed in the microbiology laboratory of King Saud University, Riyadh, KSA.

Sputum specimens (~10 ml) were collected in clean, sterile, leak-proof, disposable containers and transported to the laboratory in sealed transport specimen bags. Those samples that were not processed on the same day were refrigerated in order to avoid fast-growing contaminating bacteria. For the purpose of this study, only one of the three early sputum specimens of the subjects was investigated. The remaining specimens were tested by the respective infectious laboratory centers. All the work was done in the TB processing lab with good ventilation as recommended by the Centers for Disease Control. The laboratory was constructed to maintain a "negative pressure" system in which the air flow was in one direction only, from the clean area to a less clean area (processing room). This was a restricted area that allowed limited access to trained TB personnel only.

All sputum specimens underwent routine procedures for digestion, decontamination and concentration procedures as per recommendations from the College of American Pathologists. Ziehl-Neelsen (ZN) acid fast staining (cold method) was done, and smears were read with light microscopy. A positive culture denoted 10-100 microorganisms/ml.

For the inoculation of Lowenstein-Jensen (LJ) medium, 0.5 ml of decontaminating sediment was injected onto the face of an LJ slope. The cap was loosely replaced, and vials were incubated at 35-37°C in a 5-10% CO_2 atmosphere for 6-8 weeks. Thereafter, they were examined weekly, for up to 8 weeks for evidence of growth. LJ slopes with evidence of growth were subjected to gram staining and acid-fast staining.

Prior to inoculation of 0.5 ml sediment into *Mycobacteria* growth indicator tube (MGiT), the following reagents were prepared: 15 ml of Bactec MGiT Growth supplement was aseptically added to lyophilized vial of BBL MGiT PANTA antibiotic mixture as per manufacturer's instructions. Tubes were entered into the Bactec MGiT 960 instrument for the distilled water, and 15 μl of extracted DNA for the recommended 42 days testing protocol at 37°C. The positive tubes were removed, stained for acid-fast bacillus (AFB) and gram stain plus sub-culture on to chocolate agar and incubated at 37°C for 2 days. MGiTs that flagged positive, but negative for AFB and bacterial contamination were promptly re-incubated within 5 h of removal and incubated for a 42-day protocol.

Primers were obtained from TolmolBiol with the following sequence: Tb1 5'-ACCAACGATGGTGTGTCCAT-3' and Tb2 5'-CTTGTCGAACCGCATACCCT-3'. In a 2 ml sterile clean tube, 5 μl of × 10 polymerase chain reaction (PCR) buffer was added, followed by 5 μl of dNTP's, 5 μl primer 1, 5 μl primer 2, 1 μl $MgCl_2$, 1.5 μl Taq polymerase, 5 μl extracted DNA and 22.5 distilled water [Table 1]. All components were gently mixed and placed into a PCR thermal cycler. The conditions for the PCR included a denaturation at 95°C for 3 min followed by 40 cycles of 94°C for 1 min (denaturation step), 55°C for 1 min (primer annealing step), and 72°C for 1 min (extension step). At the end of this program, the PCR thermal cycler was programmed for 10 min at 72°C for final DNA extension. As per the manufacturer's specification, restriction digestion was carried out in a total volume of 20 μl. In a 0.5 ml PCR tube, 2.5 μl of × 10 PCR buffer was added along with 0.2 μl of bovine serum albumin (10 ng/μl), 0.5 μl of BstEII (10 U/μl), 1.8 μl distilled water and 15 μl of extracted DNA.

Data was entered in Excel and statistical analysis performed using SPSS (version 16.0). Data were presented as a percentage for frequencies (%).

Table 1: Prevalence of suspected TB according to different methods of screening and diagnosis

Region	*n*	ZN stain	LJ culture	MGiT culture	PCR	RFLP
Riyadh	419	22	22	26	28	10
Madina	1350	2	3	3	3	7
Dammam	320	20	21	22	39	13
Jeddah	65	12	15	22	23	15
Hail	191	2	2	4	3	1
Qassim	104	5	5	4	5	1
Jazan	280	2	4	4	5	0.7
Taif	675	4	4	4	4	0.4
Total	3404	7	8	8	10	11

Data presented in percentage. TB: Tuberculosis; ZN: Ziehl–Neelsen; LJ: Lowenstein-Jensen; MGiT: *Mycobacteria* growth indicator tube; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism

RESULTS

A total of 3404 sputum samples were collected from suspected pulmonary TB patients. The samples were collected from eight locations across the KSA: Riyadh Hail (191 samples), Qassim (104 samples), Jazan (280 samples), and Taif (675 samples). This was done continuously until the total sample number required was achieved. This covered a period of 3 years during which 3404 samples were collected from all areas.

Table 1 shows the prevalence of suspected TB in the different regions. Differences in prevalence were apparent according to the different methods, with restriction fragment length polymorphism (RFLP) showing the lowest number of TB cases of all the methods. The ZN stain, LJ culture, MGiT culture, and PCR methods indicated that the prevalence of TB was highest in the regions of Riyadh and Dammam [Table 1]. However, by the RFLP method the prevalence of TB was highest in Jeddah and Dammam. Figure 1 shows the mapping of TB in Saudi Arabia. The PCR showed a higher detection rate than all other techniques as indicated here. Using the ZN stain, it was 6.64%, with MGiT culture it was 8.34%, 7.7% with LJ culture and 10.46% with PCR technique.

DISCUSSION

Polymerase chain reaction is known to be the fastest and the most sensitive method for diagnosing mycobacterial infections and identifying the etiologic mycobacterial species so that the appropriate therapy could be administered. Gopinath and Singh reported that multiplex PCR test showed the highest detection (77.4%); it was 34.4% on LJ culture and 20% on ZN smear.[7] Somoskövi *et al*. reported a recovery of 96.4% of MTB by using Bactec MGiT 960 liquid medium as compared to 92.7% with Bactec 12B liquid medium and 81.8% with LJ medium.[8]

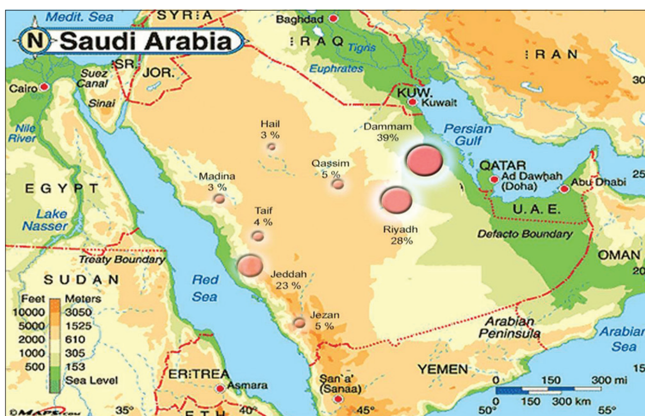

Figure 1: Mapping of *Mycobacterium tuberculosis* prevalence rate percent in Saudi Arabia

The time of recovery for the above tests was reported as 12.6 days, 13.8 days, and 20.1 days, respectively. PCR was found to be the most rapid, most sensitive and specific detecting method in all of the 150 pulmonary TB cases studied with no false positive or negative results. In contrast, MGiT 960 yielded a result of 90% followed by fast plaque assay of 76.6%, LJ culture method was 73.3% and microscopy with only 60%.[9] The data presented in this study corroborated previous findings which showed that compared to conventional methods, the PCR was the most rapid, most sensitive and accurate method for the detection and identification of MTB complex.

Percent positive results of ZN stain, LJ culture, MGiT positive, and PCR were found to be different in various regions of Saudi Arabia from where these samples were collected. Of the cities studied, Riyadh and Dammam had the highest number of positive cases. These results contradicted earlier studies which showed that the highest percentages of TB positive cases were in the Jeddah region,[10] but the findings of the present study cannot supersede previous observations until a more balanced distribution of sample size for the different regions has been done. Further, the data showed that Hail and Qassim regions have the lowest contribution of positivity rate by ZN stain, LJ culture, MGiT, and PCR techniques. The prevalence rate of TB in this study agreed with Daniel's results which confirmed that the PCR technique had the highest sensitivity in detecting MTB with 10.46% positivity rate, while ZN methods showed the lowest sensitivity with 6.64% positivity rate, and the MGiT and LJ culture showed results that were fairly close: 8.34% and 7.7%, respectively.[11] The fast results obtained by PCR with high sensitivity and specificity in the diagnosis of MTB infection compared with other tests including the biochemical methods were indicative of the highest sensitivity as in previous reports.[12] With the use of PCR, it was possible to detect MTB in samples, which were either smear or culture negative. Besides this is the speed of detection as the test can be finished in 1 day as opposed to the several days required by other methods for completion.[13]

Based on this and other studies,[14] the use of PCR for the molecular diagnosis of TB has clinical implications that may be of benefit to clinicians to help make an accurate diagnosis. In the present study, the PCR method gave a higher percentage of TB positive results as compared to other methods. However, the recent use of MGiT 960 system has made major improvements in *Mycobacteria* culture by making the detection of *Mycobacteria* much faster.[15,16] Bactec system in conjunction with P-nitro-acetylamino-B-hydroxypropiophenone have been reported to be very specific in distinguishing MTB complex

from nonTB *Mycobacteria*, but they require approximately 6 days before the results can be interpreted.[17,18]

The exclusion of inhibitory factors remains an obstacle for the eventual use of PCR as a mainstay method for routine diagnosis in clinical settings. A possible solution to this problem may be internal controls to balance and compensate for individual variations for possible contaminants and interference.[19,20]

CONCLUSION

The conventional tests for the screening and detection of MTB culture and microscopy are undoubtedly much cheaper than the PCR system. Nevertheless, these traditional methods are better in being more sensitive and faster as opposed to PCR methods. However, the adoption of accurate and precise method such as PCR and RFLP may be worthwhile, for, despite the low cost of conventional methods, patients have to contend with a lengthy stay in hospital waiting for confirmation of MTB diagnosis.

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