Hospital based prospective observational case study to evaluate the prevalence of diabetes mellitus among tuberculosis patients in a tertiary care hospital, in India
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**Background**: Diabetes mellitus (DM) is recognised as an important risk factor to tuberculosis (TB). India has high TB burden, along with rising DM prevalence. The recent change in lifestyle of people of India and westernization of food habits has contributed much to make India the diabetes capital of world. Rapid urbanization has resulted in crowding of cities and has led to rapid spread of infections. So in a majorly immunocompromised India because of DM, TB is a common infectious disease. Thus DM and TB affect each other. In both the diseases the number of cases reported at tertiary care centres is just the tip of iceberg. We conducted an observational study at, Apollo Hospital, Hyderabad, India to look for prevalence of TB among DM patients.

**Methods & Materials**: Patients older than 18 years with TB and not otherwise immunocompromised were considered for the study. All sputum positive, sputum negative and extra-pulmonary cases currently on anti-tuberculosis treatment and newly diagnosed were included in the study that were admitted as inpatient or reported as outpatient at Apollo Hospital in department of Medicine. TB patients were screened for DM through a thorough history, detailed examination and lab investigations.

**Results**: Seventyfour patients met the criteria and were included in the study. In our study 24 of total 74 i.e.(32.43%) study patients were found to be diabetic; mean age was 46 ± 17.8, (males 48.5 ± 17.4 and females 44.3 ± 19.5); 61% were male. Among the diabetic population 80% were male (p value 0.0407). 11 of 24 DM patients were newly diagnosed which is 46% and 13 patients (54%) were known diabetics. 35 patient (47%) were suffering from pulmonary tuberculosis and 39 (53%) from extra-pulmonary tuberculosis.

**Conclusion**: High prevalence of DM among TB patients was found in or study at a tertiary care hospital in India. The prevalence of DM was more in patients with pulmonary TB, among males and patients with history of smoking. We recommend screening for DM among people with TB and vice versa, in a country like India with a high double burden of disease.

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Role of PCR for diagnosing male genital tuberculosis
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**Background**: Diagnosis of male genital tuberculosis is difficult because clinical features and imaging findings are non-specific and conventional laboratory methods are time-consuming and almost non-contributory. It is important to have the diagnostic method that has high sensitivity and specificity. Hence, this study was done to see the utility of tissue and semen PCR in diagnosis of male genital tuberculosis and its comparison with histopathological examination (HPE).

**Methods & Materials**: A prospective observational study was done from Jan 2006-Dec 2014 in Department of Urology and Microbiology of Kasturba Medical College, Manipal. 74 tissue samples (Epididymis 49, Prostate 20 and Periurethral 5) of suspected cases of male genital TB were processed for both HPE and PCR. 15 semen samples from patients with hematospерmia were processed for only PCR.

**Results**: 32 tissue samples (22 epididymis and 10 prostate tissues) were positive for both HPE and PCR whereas 36 samples were negative for both tests. False positive and false negativity was observed in 4(5.4%) and 2(2.7%) samples respectively. Considering HPE as gold standard, PCR showed the sensitivity and specificity as 88.9% and 94.7% respectively with kappa agreement as 0.8. Time taken for PCR results was about 3.1 days as compare to 6.2 days for HPE. Semen PCR showed positivity for 4 samples (26.7%).

**Conclusion**: Tissue PCR is a sensitive and specific method for obtaining early and timely diagnosis of male genital tuberculosis. Semen PCR adds qualitative benefit for diagnosing tuberculosis in male genital tract.

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Intranasal delivery of antituberculosis agents in a murine tuberculosis model
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**Background**: The use of aerosol delivery of antimycobacterial agents for the therapy of tuberculosis in mice has not been well
studied. Aerosol therapy in mice is problematic due to the high cost of the required apparatus. The purpose of this study was to explore the efficacy of intranasal (IN) administration (as a surrogate for aerosol delivery) of isoniazid (INH) and rifalazil (RZL) in a murine tuberculosis model compared to oral delivery.

Methods & Materials: Six week old female Balb/c mice (purchased from Charles River Laboratories, Wilmington, MA) were infected intranasally with about 10^3 CFU of *Mycobacterium tuberculosis* (MTB) ATCC 27294 (H37Rv) for experiment 1 or about 10^6 CFU of MTB ATCC 35801 (Erdman) for experiment 2. One week post infection mice in experiment 1 were treated with INH 5mg/kg orally by gavage or 5mg/kg IN for 3 days and mice in experiment 2 were treated with RZL 5 mg/kg orally by gavage 5 days/week or 5mg/kg IN Mon, Wed, and Fri for 2 weeks. At the initiation of therapy in each experiment a group of mice (early controls) were euthanized by CO2 inhalation and their lungs were collected. At the completion of therapy an un-treated group of mice, late controls (LC), and treated mice were euthanized. Mycobacterial loads in right lungs were measured by serial dilution and plating on Middlebrook 7H10 agar plates.

Results: The mycobacterial loads (log CFU) for the EC, LC, INH oral and INH IN were 3.34, 4.49, 2.94, and 2.82 respectively (exp. 1). The mycobacterial loads for the EC, LC, RZL oral, and RZL IN were 6.26, 8.93, 4.30, and 4.58 respectively (exp. 2). The LC group was euthanized 3 days early due to their advanced illness. IN delivery of INH and RIF was significantly better than the untreated late controls.

Conclusion: The activities of INH and RZL by IN delivery in these experiments suggest that other agents that cannot be given orally could be evaluated for their potential therapeutic activities by IN administration.

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Type: Poster Presentation

Real-time PCR of whole blood specimens transported in PrimeStore MTM® to detect and monitor MTB bacteremia

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Background: *Mycobacterium tuberculosis* (MTB) is an important cause of bacteremia and sepsis in HIV patients residing in sub-Saharan Africa. Many patients with MTB sepsis go undiagnosed and die within 18 days of presentation, making culture inadequate for detecting MTB in the blood. Objective: To determine the feasibility of ambient temperature transport of blood in PrimeStore MTM® to a distant lab for real-time PCR detection of MTB bacteremia and to monitor clearance of MTB from the blood after therapy.

Methods & Materials: BALB/c female mice were injected intravenously with 0.2 mls of ethanol killed MTB (approximately 10^5 CFU/mL). Two anti-MTB opsonophagocytic bactericidal MABs were used to simulate treatment of MTB sepsis. Mouse monoclonal antibodies (MAB LHN-AB9 or LHN-GG9) or control were given IP using 0.3 mls of sterile PBS 24 hours before MTB challenge. To monitor MTB in the blood, mice were bled at 3 time points: immediately after injection with MTB and again at 4 and 24 hours before MTB challenge. Collected blood was placed into citrate tubes and 0.1ml was transferred into PrimeStore MTM®. Samples were transported at ambient temperature from Gaithersburg, MD to San Antonio, TX. DNA was extracted from blood in PrimeStore MTM® and replicate real-time polymerase chain reactions (PCR) were performed using PrimeMix® MTB Complex on an ABI 7500 Instrument.

Results: Blood PCRs on specimens obtained 15 minutes after MTB challenge were positive with an average CT value of 29.8 (range 29.2-30.6). Mice treated with PBS control had MTB detected in the blood by PCR at all time points (at 15 min, 4 and 24 hours post challenge). Mice given anti-TB opsonic MABs cleared the MTB from the blood either by 4 or 24 hours (CT = 40).

Conclusion: Blood specimens were efficiently transported to a central lab to detect MTB bacteremia by PCR. In addition, PCR may be useful to monitor patient treatment, similar to viral load testing for HIV. Using PrimeStore MTM® to ship specimens safely and rapidly at ambient temperature to a regional facility for PCR analysis may provide rural hospitals in sub-Saharan Africa the opportunity to diagnose MTB sepsis and monitor treatment.

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GeneXpert detection of mycobacterium tuberculosis from sputum collected and transported in a molecular transport medium


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Background: The Cepheid GeneXpert (Xpert) System is a WHO-endorsed, widely utilized molecular diagnostic platform for *Mycobacterium tuberculosis* (MTB) and rifampin (RIF) detection. In spite of global success, several challenges remain, especially the need for safe, cost-effective transport/shipping, and improved MTB detection sensitivity. PrimeStore Molecular Transport Medium® (PS-MTM) was developed for specimen inactivation and DNA/RNA stabilization at ambient temperature for downstream molecular applications. Aims: Xpert MTB-RIF assay was compared to real-time PCR to evaluate detection of MTB and rifampin (RIF) in PS-MTM or PBS control medium over a dynamic concentration range. Furthermore, Xpert MTB/RIF detection was performed using clinical smear-positive sputum collected from swabs in PS-MTM and PBS control, or processed as raw sputum.