RESEARCH ARTICLE

CBNAAT: A STUDY FROM ACSR MEDICAL COLLEGE, NELLORE

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Abstract

Tuberculosis and Human Immunodeficiency virus have been the two leading causes of infectious diseases associated with mortality worldwide. HIV infection is a well recognised risk factor for both activation of initial infection and reactivation of latent infection. This study was done to assess co-prevalence of TB among HIV infection using GeneXpert MTB/RIF assay. Six hundred and forty three samples with symptoms suggestive of pulmonary tuberculosis were included in this study. Rifampicin resistance was studied by GeneXpert. Sputum specimens were processed for TB detection by Cartridge based nucleic acid amplification testing using Xpert MTB/Rif assay technique. A total of 643 patients with symptoms suggestive of pulmonary tuberculosis in a period of 5 months were studied. Out of these, 287 were reported positive for tuberculosis by CBNAAT. Incidence of tuberculosis in reactive females was 68% and males 60.4% and total prevalence was 28.14% in our study. Prevalence of Rifampicin resistance was 1.09%. It is important to screen MDR-TB cases especially in the immunosuppressed individuals to identify early resistance and also to prevent spread of MDR-TB and Xpert MTB/RIF is a better screening tool.

Introduction:

Presently, 1/3 of world’s population is thought to be infected with Tuberculosis. New infections occur in about 1% of the population each year. There were more than 10 million cases of active tuberculosis which resulted in 1.3 million deaths. This makes it the number one cause of death from an infectious disease. Human immunodeficiency virus (HIV) and tuberculosis individually have been among top ten causes of mortality all over the world. Patients living with HIV (PLHIV) are more likely to acquire tuberculosis than others. Tuberculosis is the leading cause of death among PLHIV. Diagnosis of tuberculosis conventionally relies on microscopy which has a sensitivity of only 40-60% under field conditions falling to a low of 20% in the presence of HIV co-infection. In HIV-TB coinfected patients, sputum is often scanty and the number of bacilli are low due to rarity of cavitary lesions or caseous necrosis. Culture and sensitivity is the gold standard for final determination and also for drug susceptibility testing. But it is time consuming and takes about 6-8 weeks for the result. CBNAAT is a recently introduced PCR based technique for detection of tuberculosis. It also detects Rifampicin resistance as it targets rpo B gene of mycobacteria. CBNAAT is a Mycobacterium tuberculosis specific automated, cartridge based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real-time PCR, providing results within 100 minutes.

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This study was carried out to evaluate the role of CBNAAT in early diagnosis of tuberculosis and detection of M tuberculosis in sputum samples.

Materials and Methods:-
The study was carried out in the RNTCP laboratory attached to the department of Microbiology, ACSR Medical College, Nellore. Sputum samples from different health centres, referred cases from District TB centres and ART centres were received for examination. Study period is from February to June. A total of 643 sputum samples were collected from suspected cases of tuberculosis. All the sputum samples were collected in special Falcon tubes after thorough rinsing of oral cavity with clean water. All the Samples collected were subjected to CBNAAT.

The specimens were processed by Xpert MTB/Rif assay following the guidelines document provided by central TB division. Rpo B gene of M tuberculosis was extracted amplified as it accounts for more than 95% of mutations associated with Rifampicin resistance. This ensures high degree of specificity by usage of three specific primers and 5 unique molecular probes. Results can be distinguished as MTB detected, MTB not detected, RIF resistance detected, RIF resistance not detected with the help of positive beacons and their detection timing, sample processing controls.

Manual step involved are adding sample reagent to liquefy sputum and sample loading in the cartridge.

Results and Discussion:-
A total number of 643 sputum samples were collected from suspected cases of pulmonary tuberculosis and were tested on Gene Xpert MTB/Rif assay. Of the samples tested, 287(44.63%) were found to be positive by CBNAAT.

Total number of males tested were 411 (63.92%) and females were 232 (36.08%). Of 411 males tested, 281 (68.37) were reactive for HIV and 165 (71.12) of 232 females were reactive.

A number of 187(65.15%) male and 100(34.85%) female patients were positive among the 287 CBNAAT positives. Among the 187 positive males, 113(60.43%) were reactive and among the 100 positive females, 68(68%) were reactive for HIV. Total number number of HIV-TB was 181 in a total of 643 sputum samples tested. Prevalence of HIV-TB was 28.14% in our study and this coincides with a study by BG Williams et al., mentioning the incidence of HIV-TB prevalence in high prevalence states of South India with a rate between 18-45%.

Of the 287 CBNAAT positive cases, 181(63%) were reactive for HIV and of these, a total of 113(63%) males and 68(37%) were females. This male predominance may be accounted by their behavioural risks and this coincides with the studies by Prem Prakash Gupta et al., (66.6% and 33.3%), Desai K et al., (64.86% males) and Sumangala V. et al., (67% and 33%).

We can also detect Rifampicin resistance in sputum positive cases and helps us to advice correct line of treatment for the patient. In our study, Seven patients were identified to be resistant to Rifampicin. Five were males and two females and of these, 2 males were reactive to HIV. Prevalence of Rifampicin resistance was noted to be 1.09% in our study which correlates with the study by D.S. Sowjanya et al., where it was 1.9%.

Conclusion:-
Tuberculosis is the most common opportunistic infection in HIV infected individuals. In endemic areas like India, it is important to screen MDR-TB cases especially in the immunosuppressed individuals to identify early resistance and also to prevent the spread of MDR-TB. Xpert MTB/RIF is a better screening tool for simultaneous detection of MTB and Rif resistance in a shorter period of time and this would help improve early recognition and prevention of MDR-TB and its further transmission.

Tables:-
Table i: Total number of cases tested by cbnaat.

<table>
<thead>
<tr>
<th>Month</th>
<th>CBNAAT tested</th>
<th>CBNAAT positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Male</td>
</tr>
<tr>
<td>February</td>
<td>101</td>
<td>60</td>
</tr>
<tr>
<td>March</td>
<td>159</td>
<td>94</td>
</tr>
</tbody>
</table>
Table ii:- Reactive cases positive for cbnaat.

<table>
<thead>
<tr>
<th>Month</th>
<th>Reactive cases</th>
<th>Cbnaat positive</th>
<th>Rif resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>February</td>
<td>45</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>March</td>
<td>53</td>
<td>42</td>
<td>8</td>
</tr>
<tr>
<td>April</td>
<td>62</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>May</td>
<td>63</td>
<td>52</td>
<td>38</td>
</tr>
<tr>
<td>June</td>
<td>58</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>281</td>
<td>165</td>
<td>113</td>
</tr>
</tbody>
</table>

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References:-