Original Article

Microbiological Study of TB/HIV Co-infection In Relation to CD4 Count.

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ABSTRACT

Individuals with HIV infection are at increased risk for tuberculosis (TB). The altered CD4 T-cell homeostasis induced by HIV infection may play a key role in the development of tuberculosis in HIV-infected patients. Among total 961 subjects with HIV infection studied, 308 (32.055) were found positive for TB. Out of this 244 (25.39%) were positive for pulmonary TB (HIV+TB+) while extra pulmonary TB was found in 64 (6.66%) HIV patients. The mean CD4 count for extra-pulmonary TB was found to be 104.89 ±47.09 cells/μL, the mean CD4 count for pulmonary TB was 198.52±32.25 cells/μL, while the cumulative TB mean CD4 count was found to be 151.71 ± 72.62 cells/μL. In this retrospective analysis, lymphocyte profiles (CD4 counts) of subjects infected with HIV, with or without TB, were evaluated. A statistically significant difference (p = 0.01) was found in the median CD4+ counts between the HIV+TB- (269.80 cells/μL) and HIV+TB+ (151.71 cells/μL) groups. The results of this study proved that, the lower the CD4 count range, the higher was the risk of developing pulmonary TB and higher was the incidence, but when CD4 count fell below 200 cells/μL, even the risk of developing extra-pulmonary TB was noted. This was proved by finding of 40.25 % of total TB cases were with <100 CD4 count profile, followed by 31.81% of cases were with CD4 count <200 in the present study. Other 300 subjects with RTI but without HIV so immunocompetent subject with normal CD4 count profile were also screened for TB, and only 29 patients (09.67%) were found with open pulmonary TB, when this was compared with HIV positive patients the probability was found to be less than 0.02, which again proves itself that with lowering of CD4 count profile in HIV infected patients the risk of developing TB increases which was directly reflected in increased incidence.

1. Introduction

The clinical course of human immunodeficiency virus (HIV) disease and pattern of opportunistic infections varies from patient to patient and from country to country. The clinical profile of HIV disease in India includes a wide range of conditions like tuberculosis, cryptococcal meningitis, popular pruritic eruptions, and cytomegalovirus retinitis, among others. Tuberculosis is the most common opportunistic infection in Indian patients with HIV. Occurrence of various AIDS-associated illnesses determines disease progression. Mean survival time of Indian patients after diagnosis of HIV is 92 months.[1]

India has the largest number of tuberculosis (TB) cases in the world. India shoulders about 14 million cases of TB and it is estimated that about 1.8 million incident cases of TB occur in India every year of which 0.82 million are highly infectious smear positive cases.[2]

The increasing rate of human immunodeficiency virus (HIV) infection in many countries has had an impact on tuberculosis (TB) epidemiology. While TB prevalence has remained stable, TB incidence continues to rise, especially in countries most severely

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affected by the HIV epidemic as well as those facing political turmoil, migration, poverty and unemployment and where intravenous drug abuse is rampant. HIV is the most important known risk factor that promotes progression to active TB in people with Mycobacterium tuberculosis infection (TB/HIV A Clinical Manual 2004).

The lifetime risk of tuberculosis in immunocompetent persons is 5% to 10%, but in HIV positive individuals, there is a 5% to 15% annual risk of developing active TB disease. [3] WHO estimated 9.2 million new cases of TB globally in 2006 (139 per 100,000); of whom 7,09,000 (7.7%) were HIV positive. India, China, Indonesia, South Africa and Nigeria rank first to fifth in terms of incident TB cases. In India, there were 2.5 million people living with HIV and AIDS (PLWHA) at the end of 2007 while the incidence of TB was approximately 1.8 million cases per year. [4] This was found in a survey carried out among new tuberculosis patients by the Revised National TB control Program (RNTCP) in 2007, HIV sero-prevalence.

There exists a synergistic relationship between TB and HIV. The interface between TB and HIV is increased in countries like India where both TB and HIV infection are maximally prevalent in people of 15-49 years of age. [5] The association between HIV and tuberculosis present an immediate and grave public health and socioeconomics treat in developing countries. [6]

Persons infected with Tubercle bacilli have about a 10% chance of developing tuberculosis during the remainder of their lives: Thus, they have a less than 0.5% chance of developing overt disease annually. [7] while 10% of persons infected by both TB and HIV develop tuberculosis disease annually. [8] The implication of HIV infection is that it activates dormant tuberculosis to rapid disease progression of tuberculosis and death. [9] In fact, tuberculosis is now the most common opportunistic infection in patients from developing countries who die from AIDS. [10] Reports show that active tuberculosis increases the morbidity and fatality of HIV-infected person and about one-third die of tuberculosis. [8]

The largest increase in tuberculosis has occurred in locations and demographic groups with the highest HIV prevalence, which suggests that the epidemic of HIV is at least partially responsible for the increase of tuberculosis. [11] There is evidence that immune responses in tuberculosis and in other infection induce cytokines that enhance the replication of HIV and this drives the patient into full picture of AIDS. [12]

2. Materials & Methods

The present study got approval from the ethical committee of Government Medical College, Surat and even got permission of GSACS, Ahmedabad.

A predesigned and pretested questionnaire was used to collect data on socio-demographic profile. Blood samples of these subjects were tested for HIV. The HIV-infected patients were all diagnosed as HIV reactive as per the NACO guidelines.(2010). [13] In the patients found HIV sero-positive even, CD4 count was calculated on FACScnt, by flowcytometry method (Becton Dickinson) method from their blood samples.

Three consecutive early morning sputum samples were collected and even samples were concentrated before reporting negative for AFB. From 961 HIV infected patients and 300 HIV sero-negative subjects who had complained of cough and fever for more than one week sputa samples were collected in a sterile wide-mouthed container. The quality of the expectorated sputum was assessed both by macroscopic and microscopic examination. Any sample that was thin, watery and with no purulent matter was considered unsuitable for further processing. Barlett’s scoring method was used for microscopic evaluation of the expectorated sputum. [14] A sputum was considered unsuitable if it had a final score of 0 or less. All unsuitable specimens were discarded and a repeat specimen was collected.

Case Definition for T:

Cases were defined as patients with both HIV sero-positive as well as having complaints of cough and fever for more than one week or in other words suffering from respiratory tract infections (RTI) at the time of sputum and data collection. One patient was included only once.

Definition for C1(Control Group):

C1 Control group was defined as patients with respiratory tract infection (RTI) but sero-negative for HIV at the time of sample and data collection.

Sputum smear microscopy: The most frequent method of TB detection involved microscopic examination of sputum for acid-fast bacilli (AFB) [15]. Microscopy had the advantage of being inexpensive, relatively rapid to perform, and specific in most settings. However, to be considered smear positive a specimen needs to contain approximately 105 mycobacteria per milliliter. The sensitivity of sputum microscopy in HIV infection ranges from 43 to 51 per cent, [16] and in many resource-limited settings with high rates of co-infection, the sensitivity may be much lower. [17] Methods that improved speed or sensitivity include fluorescence microscopy [18] and alternative specimen processing methods, such as concentration, bleach sedimentation and same-day sputum collection [so called front loading strategies].[18,19] Any procedure for digestion or liquefaction followed by centrifugation, prolonged gravity sedimentation, or filtration increased sensitivity by 13 to 33 per cent over direct microscopy, when culture was used as the reference standard. [18]

Growth based detection: Culture of Mycobacterium tuberculosis is much more sensitive than smear microscopy and has been recommended to assist in the diagnosis of TB in HIV-infected individuals.[20] But in the present study it had been used in the limited number of the patients, which were clinically suggestive of pulmonary TB but negative by ZN staining even after concentration of sputa samples. Culture also allows subsequent strain characterization and drug susceptibility tests. The traditional method of inoculating solid medium such as the Lowenstein-Jenson (L-J) medium or Middlebrook medium is sensitive but slow, as growth may not be visible until after 6-8 wk of incubation. This results in delay in initiation of therapy, with detrimental effects on outcome of HIV TB co-infected patients. Automated liquid culture systems detected growth of mycobacteria within 1-2 wk by bacterial carbon dioxide production or oxygen consumption with radiometric sensors (BACTEC 460 TB; Becton Dickinson Diagnostic Instruments Systems, USA), fluorescent sensors [BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960; Becton Dickinson Diagnostic Instruments Systems], colorimetric sensors (MB/ BacT system; Organon Teknika), pressure sensors (ESP culture system II; Difco Laboratories, USA), or redox reagents, such as Alamar blue.[21,22,23]
3. Results

In the present study all samples were examined by ZN staining. But culture on LJ was tried only with 15 sputum samples which were clinically strongly suggestive of TB, but even after concentration of sputa got negative report for AFB. Out of 15 sputum samples cultured on LJ medium only 13 samples showed growth. 197 sputa were found positive for AFB and not yielded any other fungal or bacterial growth subsequently while 35 sputa were found positive with subsequent growth of either yeast or other bacteria. In the cases of pure pulmonary TB, 12 sputa were only culture positive and in mixed pulmonary TB cases, in only one case it found positive by culture method, after getting negative report by ZN-staining. In the present study, 64 extra-pulmonary TB cases were found, so totally in the present study pure 209 pulmonary TB cases and 35 mixed pulmonary TB cases were found, so totally 244 pulmonary cases while more 64 cases of extra pulmonary cases were found. So, total 308 TB cases were recorded and analyzed.

Table 1: Prevalence of pathogenic bacterial & Fungal isolates from both HIV seropositive(T) and HIV seronegative(C1) groups.

<table>
<thead>
<tr>
<th>Identified Pathogens</th>
<th>HIV +VE RTI +VE (T) patients (n=961)</th>
<th>HIV -VE RTI +VE (C1) patients (n=300)</th>
<th>X² Values</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>%</td>
<td>(n).</td>
<td>%</td>
</tr>
<tr>
<td>M. tuberculosis-infec.</td>
<td>209</td>
<td>21.75</td>
<td>27</td>
<td>9.00</td>
</tr>
<tr>
<td>Other bacteria</td>
<td>177</td>
<td>18.41</td>
<td>37</td>
<td>12.33</td>
</tr>
<tr>
<td>Fungal agents</td>
<td>113</td>
<td>11.75</td>
<td>04</td>
<td>1.33</td>
</tr>
<tr>
<td>Poly-Microbial</td>
<td>80</td>
<td>8.32</td>
<td>7</td>
<td>2.33</td>
</tr>
<tr>
<td>Total No. of Pathogens</td>
<td>579</td>
<td>60.25</td>
<td>73</td>
<td>24.33</td>
</tr>
</tbody>
</table>

Table 2: Polymicrobial fungal isolates from patients of both the groups.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>HIV +VE RTI +VE (T)</th>
<th>HIV -VE RTI +VE (C1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV +VE RTI +VE (T)</td>
<td>(n)</td>
<td>%</td>
</tr>
<tr>
<td>M. tuberculosis + Candida (NCAC)</td>
<td>17</td>
<td>1.77</td>
</tr>
<tr>
<td>M. tuberculosis + Candida albicans</td>
<td>6</td>
<td>0.62</td>
</tr>
<tr>
<td>M. tuberculosis + S. pneumonia</td>
<td>12</td>
<td>1.25</td>
</tr>
<tr>
<td>Total Mixed Pulmonary TB</td>
<td>35</td>
<td>2.39</td>
</tr>
<tr>
<td>Total PTB(Pure +Mixed)</td>
<td>(209+35) 244</td>
<td>25.39</td>
</tr>
</tbody>
</table>
Table 3: Distribution of TB Patients of T Group in various CD4 Ranges.

<table>
<thead>
<tr>
<th>No</th>
<th>CD4 Ranges cells/μL</th>
<th>Number of HIV patients in the different CD4 Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB - Ve</td>
<td>PTB +ve</td>
</tr>
<tr>
<td>1</td>
<td>0-99</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>100-199</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>200-299</td>
<td>131</td>
</tr>
<tr>
<td>4</td>
<td>300-399</td>
<td>157</td>
</tr>
<tr>
<td>5</td>
<td>400-499</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>500-599</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>600-699</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>700-799</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>800-899</td>
<td>34</td>
</tr>
<tr>
<td>10</td>
<td>900-999</td>
<td>05</td>
</tr>
<tr>
<td>11</td>
<td>1000-1099</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>1100-1199</td>
<td>03</td>
</tr>
<tr>
<td>13</td>
<td>1200-1299</td>
<td>11</td>
</tr>
<tr>
<td>14</td>
<td>1300-1399</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>Total</td>
<td>653</td>
</tr>
</tbody>
</table>

In the present study sputum positivity for acid fast bacilli was detected in 209 (21.75 %) RTI patients from the HIV sero-positive patients of group T, while it was in 27 (09.33 %) patients from the HIV sero-negative patients of group C1(Table 1). So, when calculated Probability value P for both the HIV sero-positive (T) and HIV sero-negative (C1) group, it was found very significant with probability (P) value 0.02.

In present study the prevalence of MTB was found to be about 10.00 % (27/300) in HIV sero-negative patients. In the study of VShailaja et al.,(2004), they found the prevalence to be 10 (3/30) % of MTB in HIV sero-negative patients in their study, which is almost same (9%) with our present study MTB prevalence in HIV sero-negative patients.

Discussion

Tuberculosis (TB) ranks as the most common infection seen in the developing countries. About 55-89% of AIDS cases in India, were found to be suffering from extensive pulmonary TB.[24] In the present study 30.80% (296/961) cases with M.tuberculosis infection were detected from the HIV sero-positive patients of group T, in the other study of Sangeeta Pate et al. (1911) from Vadodara,[25] almost the same geographical region but they found M. tuberculosis in 60.5% (23/38), the reasons for almost double prevalence of MTB in their study must be due to their different methodology of culture method in all the samples on LJ Medium for detection of TB cases, which is naturally much more sensitive method for diagnosing TB then the Zeel-Nelson staining method[ZN staining] with sensitivity of 45-51% [16] used in the present study. In another study by VVShailaja et al.,(2004) from Hyderabad,[26] they found almost the same prevalence of 28% for MTB in their study even though they had used same culture method on LJ Medium for detection of TB cases in their study. Moreover, Aruna Aggarwal et al.,(2005) also found almost the same prevalence of 31.75% of MTB in their study from HIV infected patients.[27]

Even in the study of Kumaraasamy et al(2005) conducted at YRG Care, Chennai, [1] TB was the most common opportunistic infection, with pulmonary TB affecting 35% of the HIV-positive group and extrapulmonary TB in 11% of them. In the present study 244 (25.39%) cases of pulmonary TB were found while 64 (6.66 %) cases were of extra-pulmonary TB.

In the present study, Candida(NCAC) were the most common isolate from TB/HIV patients and had been isolated from 17(1.77%) patients followed by S pneumonia which were also isolated from 12 (1.25%) patients of TB/HIV co-infection. While C. albicans was isolated only from 6 (0.62%) patients. When we compared mixed infections in HIV infected patients it was in 35 (2.39 %) patients, while they were seen only in 2(0.66%) in HIV non-infected patients. Candida species are often found in sputum specimens suspected of tuberculosis. Their role as a possible cause of pulmonary disease is a frequent consideration, particularly in patients receiving immunosuppressive or long-standing antimicrobial therapy.[28] Though Candida is not a significant clinical factor in pulmonary diseases, it causes serious complication as coinfection with other chronic illness.[29]

The depletion of CD4+ T cells, which is a main feature of AIDS, is certainly an important contributor to the increased risk of reactivation of latent TB and susceptibility to new M. tuberculosis infection. There is also some evidence that CD8+ T cells play a role in the control of latent TB.[30,31] Other mechanisms reported to facilitate M. tuberculosis infection and disease in individuals with HIV are up-regulation of M. tuberculosis entry receptors on macrophages, [32] HIV manipulation of macrophage bactericidal pathways, [33] deregulated chemotaxis, [34] and a tipped Th1/Th2 balance.[35] It has also been shown that HIV impairs tumor necrosis factor (TNF)-mediated macrophage apoptotic response to M. tuberculosis and thus facilitates bacterial survival[36].
In the latent phase of TB, the bacteria are not completely eradicated despite a seemingly robust Th1 immune response. A failure or an alteration of the quality or levels of the protective adaptive immune responses or of the cross-talk with innate immune responses leads to reactivation of infection. Several immune mechanisms, such as increased levels of Foxp3+ Treg cells, increased production of IL-27, [38] TGF-β, [39] PGE-2, [40] SOCS1, or the decoy receptor D6, [41] or diminished levels of IFN-γ, TNF, and poly-functional specific T cells, are believed to play a role in such reactivation. Many of these factors, such as SOCS1 or IL-27, down-regulate the IFN-γ/IL-12 axis, thereby impairing bacterial control, while others, such as the D6 decoy receptor, are mainly anti-inflammatory, but may indirectly inhibit efficient bacterial clearance. Some of these mechanisms may also underlie HIV-infected patients' increased susceptibility to active TB.

Granulomas are organized cellular structures that constitute TB's pathologic hallmark. Mycobacteria are contained within the granuloma, which, by localizing infection and thus potentially preventing spread of the disease between hosts, probably contributes to protection. CD4+ T cells and TNF are important in maintaining granuloma organization. Granuloma formation may fail in individuals with a compromised immune system, and there are several hypotheses about how HIV exacerbates TB pathology through the manipulation of granulomas. [42] Specifically, TB patients with AIDS present a dominant granulocytic infiltrate and necrosis without the typical caseous necrosis seen in non-HIV-infected TB granulomas. This has been associated with the killing of CD4+ cells in the granuloma, probably resulting in a direct disruption of granuloma structure and abolition of the containment of infection. Cavitary lesions are seldom encountered in patients with a CD4 T-lymphocyte count <200/mm3. [43] As a result, while in the majority of adult patients TB is confined preferentially to the lungs, in HIV-infected patients TB can be a systemic disease involving multiple organs that lack well-defined granulomas and instead develop more diffuse lesions. [44] All forms of extra-pulmonary TB have been described in patients with HIV.

As mentioned earlier in the present study other 64(6.66%) more cases of extra-pulmonary TB were recorded which were having either abdominal, genitourinary, pleural TB, or even tuberculous meningitis. Out of 64 cases 61cases(95.31%) were observed below CD4 count below 200 cells/μL. Out of 64 extra-pulmonary TB cases 39(60.94%) were observed with <100 cells/μL CD4 count, while other 22(34.38%) cases were recorded <200 but >100 CD4 counts. If we count total TB cases below <100 cells/μL CD4 count, they were 124(80.25) cases out of total 158 cases with <100 cells/μL CD4 counts, i.e. 68.89%(40.25% TB cases) of the patients were observed suffering from either pulmonary TB or extra-pulmonary TB with <100 cells/μL CD4 count in the present study. When the patients with CD4 count >100 but <200 were analyzed, 98(76+22) cases out of total 194 cases, i.e. 50.52 and if we calculate only TB patients it would be 31.81%. The mean CD4 count for pulmonary TB was found to be 198.25 ± 32.52, which was threshold for TB, and that was why we detected about four fold increase in the incidences between CD4 count range of 200-299 and 100-199 cells/μL(Figure 1).

Figure 1: Distribution of All Pulmonary TB cases(244) in various CD4 Ranges

Besides, with decreasing of CD4 count, not only decreased CD4 count favored the risk of acquiring the TB. With decreasing CD4 count many other severe changes takes place in immune system of HIV patients with decreasing CD4 count, these factors favoring acquiring TB has been highlighted and been discussed in this article. In the present study not only decreased CD4 count had been tried to co-relate with increased incidences of TB, other changes taking place in the immune system were also discussed.

In macaques, SIV induces distortions in pro-inflammatory and anti-inflammatory T cell responses within the granuloma that may have significant effects on reactivation of latent TB. Reduction of T cell numbers also occurred within lung granulomas of monkeys co-infected with SIV compared with monkeys exclusively infected with TB.[42] It is important to note that besides the known increased risk of disseminated disease in adults with HIV, there is a growing recognition from prevalence surveys of sub clinically active TB infection in co-infected individuals.[45]

TB/HIV co-infection represents a novel pathogenic scenario at the global level. It constitutes a serious diagnostic and therapeutic challenge and, particularly in poor countries, weighs heavily on already strained health care budgets. It has recently been realized that the epidemiology, clinical manifestations, and management of both HIV and M. tuberculosis infections are different and far more complex in co-infected compared to mono-infected patients. However, our knowledge about the mechanisms of interaction of the two pathogens still has many gaps that need to be filled in order to develop preventive measures against the two diseases.

**Conclusion:**

This study supports the finding of other studies, suggesting that prevalence of TB in HIV infected patients is inversely proportional to CD4 count. Means, lower the CD4 count higher would be prevalence of TB in HIV infected patients. The patients of HIV may develop Extra-pulmonary TB, when their CD4 count falls below 200, which is most commonly seen with tremendously immune-deficient patients with very low CD4 count.
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