STUDY OF OPPORTUNISTIC PATHOGENS IN LOWER RESPIRATORY TRACT INFECTIONS AMONG SUBJECTS WITH ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) IN A TERTIARY CARE CENTRE OF TRIPURA

Swapan Chakma¹, Tapan Majumdar², N. G. Brajchand Singh³

¹Senior Resident, Department of Microbiology, Agartala Government Medical College and GBP Hospital, Agartala, Tripura.

²Associate Professor, Department of Microbiology, Agartala Government Medical College and GBP Hospital, Agartala, Tripura.

³Professor, Department of Microbiology, Agartala Government Medical College and GBP Hospital, Agartala, Tripura.

ABSTRACT

BACKGROUND

Organisms responsible for opportunistic infections (OIs) differ in characteristics from that of conventional communicable disease causing agents and are mainly of low virulence, or are non-virulent. OI usually sets in when there is a gradual decline of CD4 count from 500/mm³ and life threatening OIs occur in CD4 count below 200/mm³.

Aim and Objective- To study the opportunistic pathogens in Lower Respiratory Tract Infections (LRTIs) among subjects with Acquired Immune Deficiency Syndrome (AIDS) with objectives of isolation and identification of the bacterial and mycotic agents responsible for this condition, and establishing a correlation between the spectrum of opportunistic infections with CD4 count.

MATERIALS AND METHODS

Study period was from January 2014 to December 2015, two consecutive sputum samples were collected and inoculated for bacterial and fungal culture following Gram stain and KOH wet mount. Isolated colonies were identified by standard biochemical test. CD4 estimation was done by using BD FACS machine as per the manufacturer's protocol.

RESULTS

Out of the 151 cases, 70.19% (106/151) were culture positive. Among the culture positive cases, bacterial isolates were seen in 78.31% (83/106), while fungi were seen in 21.69% (23/106). The most prevalent bacterial pathogen was *Mycobacterium tuberculosis* (MTB) 51.88% (55/106), *Staphylococcus aureus* 19.81% (21/106), *Pseudomonas aeruginosa* 16.98% (18/106), *Klebsiella pneumoniae* 10.37% (11/106), *Citrobacter koseri* 7.54% (8/106) and *Citrobacter freundii* 2.83% (3/106) and in fungal pathogens the most prevalent is *Candida albicans* 11.32% (12/106) and *Candida guilliermondii* 10.37% (11/106). CD4 count was found to be below 500/μL among the culture positive study subjects.

CONCLUSION

Respiratory pathogens were found to be important opportunistic agents among HIV/AIDS positive patients. This study shows that MTB infection and Candidal infection are commonest among bacterial and fungal agents. CD4 estimation helps in early diagnosis and management of suspected LRTI patients with HIV.

KEYWORDS

HIV/AIDS, LRTI, Opportunistic Pathogens, CD4 Count.

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BACKGROUND

Organisms responsible for OI differ in characteristics from that of conventional communicable disease causing agents and are mainly of low- or non-virulent.^[1] OI occurs in individuals whose resistance has been decreased by disorders such as diabetes mellitus, HIV infection, cancer, long term antibiotics therapy or immunosuppressive drugs.^[2] Lung is a major target organ of LRTI in HIV infection. Over 98% of respiratory infections are infectious and the most frequent complications are acute bronchitis, bacterial

pneumonia and *Pneumocystis pneumoniae* (PCP).^[3] Usually HIV positive individuals have different predisposing factors like neutropenia, lymphopenia, selective T cell defects, altered monocyte macrophage function, frequent use of antibiotics for prophylaxis & treatment of various bacterial infections, lowered immunity due to low CD4 count. All these factors contribute to the development of opportunistic infections like pulmonary tuberculosis, Candidiasis, Cryptococcosis, Zygomycosis, Aspergillosis, *Pneumocystis pneumoniae*, etc.^[4] OIs involve all systems like gastrointestinal system, central nervous system, and respiratory system. Association of pulmonary infection with different CD4 counts showed that when the CD4 count is <500 cells/mm³ the person developed acute pharyngitis, bronchitis, sinusitis, pneumonia and pulmonary tuberculosis. When CD4 count is 200-500 cells/mm³ person is exposed to recurrent bacterial pneumonia. When the CD4 count is 100-200 cells/mm³ the person is exposed to pneumocystis pneumonia and disseminated tuberculosis, and when CD4 count <100 cells/mm³, person is exposed to disseminated tuberculosis and fungal pneumonia.^[5]

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Corresponding Author:

Tapan Majumdar,

Associate Professor,

Department of Microbiology,

Agartala Government Medical College and GBP Hospital,

Agartala, Tripura, India.

E-mail: drtapan1@rediffmail.com

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Also, this makes them more susceptible to bacterial infections like *Streptococcus pneumoniae*, *Salmonella spp.*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is the second most common infection after mycobacteria.^[6] Tuberculosis (TB) is much higher among people infected with HIV. Without treatment, mortality rates are high, around 70% within 10 years.^[7] In an immunocompromised individual infected with HIV, *Candida albicans* infection is the most common opportunistic fungal infection followed by *Cryptococcus neoformans*, Dermatophytes and *Aspergillus*.^[8] *Pneumocystis jirovecii*, which was one of the important OI among HIV infected patients, but rarely documented in Indian patients, except for a few occasional reports which varies from 6-10%.^[9,10,11] As per the data from Tripura State, AIDS Control Society a total number of 2000 HIV seropositive cases have been detected in Tripura. However, the spectrum of opportunistic pathogens causing respiratory tract infection in HIV individuals is not known in our setup, this study proposes the determination of aetiological agents causing opportunistic infection with special reference to respiratory tract infections and their correlation with CD4 count.

MATERIALS AND METHODS

This is a cross sectional study, done from January 2014 to December 2015 on HIV/AIDS subjects with CD4 count 500 cells/mm³ or below with features of LRTIs. Subjects with CD4 count above 500 cells/mm³, lung carcinoma and interstitial lung disease were excluded from the study. Prior to initialisation of the study institutional ethical committee approval was taken and consent form was signed by the study subjects.

Collection of Sample

Consecutive two days sputum samples were collected in a sterile wide mouth container for isolation of pathogens from 151 HIV seropositive subjects with suspected LRTIs with CD4 count $\leq$ 500 cells/ μ L in the Department of Chest and Respiratory Disease.

- a. For isolation of bacteria, the samples were transported to the laboratory and were processed for isolation and identification of bacteria by inoculation on Blood agar and MacConkey's agar and incubated at 37°C overnight. After isolation, the identification of the organisms were done by a standard biochemical test^[12,13] and antibiotic sensitivity was assessed on Mueller-Hinton agar (MHA) medium by disc diffusion method developed by Kirby and Bauer as per CLSI guideline and Sensitivity and resistance was interpreted according to the Zone size interpretative chart.^[12-14]

For Quality Control

Standard strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used during culture and antimicrobial susceptibility test.^[15]

- b. For isolation of Mycobacteria, the sputum smears were stained by ZN stain followed by inoculation onto Lowenstein-Jensen (LJ) Media with and without glycerol, incubated at 37°C for eight weeks.^[16] Species identification was done by standard biochemical test, and Antitubercular drug sensitivity test was done by

proportionate method^[17] by using H37RV reference strain which is maintained in the laboratory.

- c. For Isolation of Fungus, the samples were cultured onto each pair of SDA and SDA with 0.004% chloramphenicol and 0.05% Cycloheximide following KOH (Potassium hydroxide) mount examination. The culture tubes were incubated at 25°C and 37°C and examined daily for four weeks. Growth in the culture medium was identified by Lactophenol Cotton Blue (LPCB) mount and doubtful morphological features were confirmed by slide culture. Confirmation of *Candida* species were done by Germ tube test, Rapid urea hydrolyse test, culture on Corn Meal Agar (CMA), sugar fermentation and assimilation tests.^[18,19] Antifungal drug susceptibility test was done by disc diffusion method.^[18,19]
- d. At the end of the study period, data were compiled and results were analysed by standard statistical methods (Chi-square test) by using SPSS 15.0 version.

RESULTS

During the study period, 302 sputum samples were collected from 151 HIV seropositive subjects with CD4 count $\leq$ 500 cells/ μ L with features of LRTIs as per inclusion criteria. Age distribution of the study subject shows predominant age group is 26 – 35 years 37.74% (57/151) and among them 66.88% (101/151) were male and 33.12% (50/151) were female (Table 1 & 2).

Bacteriological Analysis

Single sputum samples, i.e. 151 samples were inoculated, of which 26.49% (40/151) showed Gram-negative Bacilli (GNB) and 13.90% (21/151) were Gram-positive Cocci (GPC). Among the GNB, 14.56% (22/151) were lactose fermenter (LF) and 11.92% (18/151) were non-lactose fermenter (NLF). Predominant LF were identified as *Klebsiella pneumoniae*, *Citrobacter koseri* and *Citrobacter freundii* and NLF isolates were *Pseudomonas aeruginosa*. Predominant GPC identified as *Staphylococcus aureus*.

Antibiogram

Overall, GPC showed sensitivity to Vancomycin, Ceftriaxone, Moxifloxacin, Ofloxacin, Azithromycin, but were resistant to Penicillin and Amoxyclav. Among GNB, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter koseri* and *Citrobacter freundii* were sensitive to third generation Cephalosporins, Quinolones, Aminoglycosides, but resistant to Ceftazidime and Cefotaxime (Table 3).

Solid culture on LJ media showed growth of Mycobacteria in 36.42% (55/151) samples, all of them identified as *Mycobacterium tuberculosis* by standard biochemical test.

Drug Susceptibility Test for MTB

All the isolates were sensitive to first line antitubercular drugs.

Isolation and Identification of Fungus

Fungus was isolated in 15.23% (23/151) samples of which 7.94% (12/151) were *Candida albicans* and 7.28% (11/151) were *Candida guilliermondii*.

Antifungal Drug Sensitivity

All the isolates were sensitive to Fluconazole, Ketoconazole, Itraconazole, Amphotericin B and Nystatin.

Age Group	No. of Subjects	Percentage (%)
16 – 25	16	10.60%
26 – 35	57	37.74%
36 – 45	54	35.76%
46 – 55	24	15.90%
Total	151	100%

Table 1. Age Group Distribution of Study Subject

Sex	Frequency	Percentage (%)
Male	101	66.88%
Female	50	33.12%
Total	151	100%

Table 2. Sex Distribution of Study Subject

Antibiotics	Organisms (Percentage of Sensitivity)				
	Klebsiella Pneumoniae (n=11)	Pseudomonas Aeruginosa (n=18)	Citrobacter Koseri (n=8)	Citrobacter Freundii (n=3)	Staphylococcus Aureus (n=21)
Amoxyclav	54.54% (6)	38.88% (7)	50% (4)	66.66% (2)	0% (0)
Meropenem	100% (11)	100% (18)	100% (8)	100% (3)	--
Amikacin	81.81% (9)	72.22% (13)	100% (8)	100% (3)	--
Ceftriaxone	72.72% (8)	44.44% (8)	87.5% (7)	66.66% (2)	90.47% (19)
Piperacillin	100% (11)	94.44% (17)	100% (8)	100% (3)	--
Gentamycin	54.54% (6)	61.11% (11)	75% (6)	100% (3)	--
Moxifloxacin	81.81% (9)	55.55% (10)	87.5% (7)	66.66% (2)	85.71% (18)
Cefotaxime	36.36% (4)	0% (0)	37.5% (3)	33.33% (1)	--
Ceftazidime	0% (0)	0% (0)	37.5% (3)	33.33% (1)	--
Vancomycin	--	--	--	--	100% (21)
Azithromycin	--	--	--	--	76.19% (16)
Penicillin	--	--	--	--	0% (0)
Ofloxacin	63.63% (7)	55.55% (10)	87.5% (7)	100% (3)	80.95% (17)

Table 3. Antibigram of the Bacterial Pathogen

DISCUSSION

LRTI is one of the most common cause of OI among the HIV seropositive subjects when CD4 count ≤ 500 cells/ μ L and these infections vary from region to region.^[20] Sociodemographic profile of the study shows most common age group 26 - 35 years followed by 36 - 45 years, 46 - 55 years and 16 - 25 years respectively and were more common in males than in females, which is very much similar to studies carried out by Adeleye et al (2008).^[21,22,23] From the study it was observed that the frequency of bacterial species responsible for the respiratory tract infections among the HIV seropositive patients were 70.19% (106/151). This is also very much similar to studies carried out by VV Shailaja et al, (2004).^[24] It also observed when CD4 counts were ≤ 500 cells/ μ L, MTB was found to be highest followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter koseri* and *Citrobacter freundii* which is very similar to studies carried out by Afessa et al, 2000 and Nuorti et al, 2000.^[25,26] Next to bacterial infection the fungi that were commonly found included *Candida albicans* and *Candida guilliermondii*, which is similar to studies carried out by Mohanthy et al, 1993^[27] from India, but *Pneumocystis jirovecii* were not isolated. This is also very similar to a study carried out by Singh YN et al, 1993; Mirda BR et al 2000; Singh A et al, 2014, India.^[9-11] No atypical Mycobacteria and no multidrug resistance MTB were isolated in these subjects by conventional method, *Streptococcus pneumoniae* and *Haemophilus influenzae* were also not isolated during the study period, which is similar to studies carried out by Pereira M, Tripathy S et al, 2005, India; and C. Panana priyadarsini, G. Narendran et al, 2011; Noskin et al, 1995 and in Ghana, Newman et al, (2006).^[28-31]

The antibiotic susceptibility profile of the isolates are in complete agreement with the study carried out by Ojo-Bola. O, Oluyeye A.O., 2014.^[32] In the present study, *Staphylococcus aureus* is mostly sensitive to Ceftriaxone, Moxifloxacin, Azithromycin, Ofloxacin and 100% sensitive to Vancomycin. The isolated Enterobacteriaceae were mostly sensitive to Meropenem, Amikacin, Piperacillin, Gentamycin, and *Pseudomonas aeruginosa* also having more or less the same sensitivity pattern like Enterobacteriaceae. Among the fungal isolates, azoles resistance was not encountered as they were all sensitive to Fluconazole, Ketoconazole, Itraconazole, Amphotericin B and Nystatin, which is very much similar to studies carried out by Deepa, Anil Kumar, Sumathi Muralidhar, Uma Banerjee et al 2015.^[33]

CONCLUSION

This study was carried out to determine the prevalence of bacterial and fungal pathogens, responsible for LRTI in HIV seropositive patients. MTB were the most predominant pathogens followed by Enterobacteriaceae and fungi isolated from the study subjects. No atypical mycobacteria or multidrug resistant mycobacteria were isolated from all these samples during the period of study from the subjects suffering from LRTI having HIV seropositive and CD4 count ≤ 500 cells/ μ L. It is interesting to observe that maximum number of the pathogens isolated from the subjects were having CD4 count < 200 cells/ μ L. The antimicrobial pattern may provide insight to physicians for treatment while awaiting for antimicrobial susceptibility test results from microbiological laboratories.

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