Print ISSN 1818-9741
Online ISSN 2091-0959
Vol. IX, No. 2, Year 2012

SAARC

(South Asian Association for Regional Cooperation)

Journal of Tuberculosis, Lung Diseases and HIV/AIDS

SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS

Vol. IX No. 2 Year 2012

Published and distributed by:

SAARC Tuberculosis and HIV/AIDS Centre (STAC)
Thimi, Bhaktapur
G.P.O. Box 9517, Kathmandu, Nepal
Tel.: 00977-01-6632601, 6632477, 6631048
Fax: 00977-1-6634379
E-mail: saarctb@mos.com.np
Website: www.saarctb.org

AIMS AND SCOPE:

The SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS is the official journal of the STAC. The Journal's main aim is the continuing education of personnel and the dissemination of the most up-to-date information in the field of tuberculosis, lung diseases and HIV/AIDS. It is devoted to dissemination of knowledge concerning various aspects of tuberculosis, lung diseases and HIV/AIDS. All articles relevant to the practice of this Journal and quality health research are published. The Journal is an appropriate forum for the publication of articles concerning the social, economic, public health, epidemiology, diagnostics, genetics etc. in the area of tuberculosis, lung diseases and HIV/AIDS. The scientific manuscripts presenting the results of public health importance are encouraged. The novel case reports which adds to the existing knowledge and consistent with the scope of Journal will be considered for publication. The Journal accepts review/mini-review, case report, short communications, and letters to editors within the scope of the journal.

DISCLAIMER:

Any opinions expressed or policies advocated do not necessarily reflect those of the STAC.

INSTRUCTIONS TO AUTHORS:

Instructions on manuscript submission can be obtained from the STAC website www.saarctb.org

FULL TEXT VERSION ONLINE:

The full text of the Journal is published online. Free access to all published issues. Address: www.saarctb.org/stacjournal.php

Print ISSN 1818-9741
Online ISSN 2091-0959

Editorial

The World Health Organization reported that of the 8.7 million incident TB cases, an estimated 0.5 million were children (defined as those aged <15 years) in 2011 and 64 000 deaths occurred among children due to TB in 2011. Tuberculosis is an important cause of morbidity and mortality in children in TB-endemic countries. The burden of child TB infection and disease represents recent and ongoing transmission in the community. For many years, maximum attention has been given to the detection and treatment of TB among adults, the prevention, diagnosis and treatment of TB among children have been relatively neglected. TB illness in children is often missed or overlooked due to non-specific symptoms and difficulties in diagnosis, such as obtaining sputum from young children. Most of the children have paucibacillary TB that is harder to diagnose with sputum smear microscopy and culture. Many children, especially younger children, are also not able to expectorate sputum. On the other hand, children develop extra-pulmonary TB (EPTB) more often than do adults. Severe and disseminated TB (e.g., TB meningitis and miliary TB) occur especially in young children (<3 years old). The diagnosis in children is largely based on the clinical features of cough, weight loss, with a history of close contact with an infectious adult TB patient. With increasing coverage of BCG vaccination, the tuberculin skin test is no longer considered a confirmatory test. Children can present with TB at any age, but the most common age is between 1 and 4 years. Case notifications of childhood TB depend on the intensity of the epidemic, the age structure of the population, the available diagnostic tools, and the extent of routine contact investigation.

In HIV infected children the risk of developing TB meningitis is very high and often results in deafness, blindness, paralysis and mental retardation. Tuberculosis and malnutrition often go together, and a child with TB disease may present as failure to gain weight with loss of energy and a cough lasting for more than three weeks. Children with TB are often poor and live in vulnerable communities where there may be a lack of access to health care. Children with latent tuberculosis infection represent the future reservoir of cases of tuberculosis. Early identification, detailed evaluation including Tuberculin skin testing of young children at risk of developing infection is a critical component of Tuberculosis control efforts. Young children living in close contact with a source case of smear-positive pulmonary TB are at particular risk of TB infection and disease. The risk of infection is greatest if the contact is close and prolonged such as the contact an infant or toddler has with a mother or other caregivers in the household. The risk of developing disease after infection is much greater for infants and young children under 5 years than it is for children aged 5 years or older. If disease does develop, it usually does so within 2 years of infection, but in infants the time-lag can be as short as a few weeks. Isoniazid preventive therapy for young children with infection who have not yet developed disease will greatly reduce the likelihood of developing TB during childhood.

HIGH HIV PREVALENCE AMONG LOW INCOME AND LABORERS IN THE DISTRICT OF PURBA MEDINIPUR, WEST BENGAL, INDIA

Biswas DK[1], Bhunia R[2], Mishra A[3]

[1] Dy. Chief Medical Officer of Health-II, District Purba Medinipur, West Bengal, India
[2] Field Epidemiology Training Programme, National Institute of Epidemiology, Chennai, India and District Tuberculosis Officer, District Hooghly, West Bengal, India
[3] District Programme Manager for HIV/AIDS, District Purba Medinipur, West Bengal, India

ABSTRACT

Introduction: There were limited data of "People Living with HIV/AIDS" (PLHA). The data of PLHA were analyzed in time, place and person and socio-economic status and recommended for prevention of HIV infection. Objective of study were to describe the PLHAs in term of time, place and person distribution and their socio-demographic status, to detect the mode of transmission of HIV and to recommend for prevention of spread HIV infection and social security.

Methodology: The information about socio-demographic status and exposure history of PLHAs since 2010 was collected. Data were analyzed by using Epi-info software.

Results: Among 329 PLHAs, 165 (50%) were men and 42 (13%) were children <14 years and they acquired HIV through blood transfusion 26 (62%). A maximum 276 (84%) PLHAs were age group 15 - 45 years. Out of 25 Community Block in district, maximum PLHAs were distributed at Tamluk Block 56 (17%) followed by Paskura 43 (13%). Of PLHAs, 246 (75%) had family income rupees ≤2000.00 per month and 138 (38%) of them were laborer and 85 (26%) home maker (house-wife). Highest 289 (88%) of PLHAs acquired HIV through heterosexual route.

Conclusion: Heterosexual transmission of HIV showed among low income and laborer. The counseling for safe sex practice, employment people and social amenities were recommended.

Key words: Human Immunodeficiency Virus, Heterosexual, Purba Medinipur

INTRODUCTION

People living with Human Immunodeficiency Virus (HIV) were 33.4 million (range 31.1 million – 35.8 million) in 2008, which was 20% higher than in 2000.[1] Around 68% of People Living with HIV/AIDS (PLHAs) were in Sub-Saharan Africa.[2] It was estimated 270,000 new HIV infections in 2010 in South and South-East Asia.[3] In India, 23.7 lakh people were living with HIV in 2009.[4] Purba Medinipur district had159 new PLHAs (in 2010). Here the prevalence of HIV in the district Purba Medinipur among the high risk group was more than 5% and among the antenatal mother was less than 1%.[5]

There was inadequate information about PLHAs in the district of Purba Medinipur, West Bengal, India. Total population of the district was 46,60,448 (census 2001).[6] The district situated at the eastern side of West Bengal and a long sea beach is situated at the southern side. Literacy rate of district was 88% and it was highest among the districts of West Bengal. The secondary data of PLHA in the district of Purba Medinipur, West Bengal, India were analyzed.

Correspondence:
Dr. Pilip Kumar Biswas
Welcome Housing, 228 Ashokegarh
Kolkata – 700 108, West Bengal, India.
E-mail: dilipbiswas29@gmail.com

METHODOLOGY

It was a descriptive study. In this study PLHAs were defined as those who had been tested positive for HIV (Rapid Test and ELESA test methods) by laboratory technician at different Integrated Counseling and Testing Centers (ICTC) during the year 2005 and 2010. There were 12 ICTC in the district.

All PLHAs identified at different ICTC in the district for study were included in the study. People of Purba Medinipur also diagnosed positive for HIV at government referral laboratories situated at Medical College Hospitals, Anti Retroviral Treatment (ART) center and Institute of Tropical Medicine, Kolkata were also included in this study. People diagnosed positive by laboratories of Non-Government Hospital and private hospital were excluded for the study.

All the PLHAs had been enrolled in a data base of "SPARSHA" a Non- Government Organization (NGO). Data of PLHA from "SPARSHA" who work with PLHAs as project of "CARE & SUPPORT" in the district were analyzed. The format of semi-structured questionnaires for collection of data were used. Questionnaires containing name, age, sex, address, socio-demographic characteristics, occupation, date of onset of symptoms and treatment history of PLHA. Social workers, working in this NGO had been engaged for collection of data.

Data of PLHAs were analyzed by of age, sex, education and socio-demographic status. The distribution of PLHAs was described according to their residential address situated at Community Blocks of district. The exposure history of patients were described. The data were analyzed as percent and proportion. Epi-info and MS excel for analysis of data were used. Data were analyzed as per family income. Those who have the family income of less than Rs: 2000/month was called as Below Poverty Line (BPL) family (when people don't have money to even their day to day expense such as food, shelter, clothing etc).

RESULTS

Thirteen percent (42/329) of PLHA was age group of 1-14 years, among them boys 74% (31/42) were maximum than the girls. Maximum PLHAs were young and adult (age15- 45 years) 276 (84%). Among them 150 (46%) age group of 15-30 years and here woman were maximum 67% (100/150) than men. Thirty eight percent (126) were age group of 31-45 years, among them men were predominant 60% (75/126) than women.

Of total 329 PLHAs, 165 (50%) were men and 164 (50%) were women (Table 1).

Among the total PLHAs, 66% (216) were illiterate to 5th standard, where maximum were female 70 % (115), they were 26% (85) home maker by profession. Labor and migrant labors were 38% (128), among them men and women were 49% (80) and 29% (48) respectively. Female sex workers were only 2% (7), but truckers and student were 10% (34) and 11% (35) respectively. Maximum 75% (246) PLHAs had the family income of rupees ≤ 2000/ per month (Table 1).

Table 1. PLHA status in the district of Purba Medinipur, 2010

Characteristics	Men (n=165)		Women (n=164)		Total (n=329)	
Age (Years)	n	%	n	%	n	%
Age Group						
1-14	31	19	11	7	42	13
15-30	50	30	100	61	150	46
31 - 45	75	46	51	31	126	38
46 - 60	8	5	0	0	8	2
> 61	1	1	2	1	3	1
Education						
Illiterate	15	9	18	11	33	10
1st–5th Std	86	52	97	59	183	56
6th - 10th Std	54	33	47	29	101	31
11th -Graduate	8	5	1	1	9	3
>Post Graduate	2	1	1	1	3	1
Occupation						
House wife	0	0	85	52	85	26
Trucker	33	20	1	1	34	10
Student	25	15	10	6	35	11
Daily laborer	51	31	46	28	97	29
Migrant laborer	29	18	2	1	31	9
Business	15	9	6	4	21	6
Services	5	3	5	3	10	3
Unemployed	7	4	2	1	9	3
Sex worker	0	0	7	4	7	2
Family Income (Rupee)						
≤ Rs: 2000	114	69	132	81	246	75
> Rs: 2000	51	31	32	20	83	25

Table 2. Exposure history of PLHA in the district of Purba Medinipur, West Bengal, India 2010

Route of transmission	Men			Women			Total		
	N=165	%	95% CI	N=164	%	95% CI	N=329	%	95% CI
Heterosexual	136	82	88 - 176	153	93	97 – 188	289	88	91 - 184
Blood Transfusion	13	8	13 - 34	5	3	7 - 31	18	5	9 – 33
Parent to Child Transmission	15	9	15 – 45	6	4	8 - 41	21	6	10 – 44
Others	1	1	3 - 60	0	0	2 - 60	1	1	2 – 60
Total	165	100		164	100		329	100	

95% Confidence Interval (CI)

Among the total PLHAs, 88% (287) had history of getting infection through heterosexual route. Female were infected through heterosexual route more than male. 5% (18) of PLHAs had history of blood transfusion having thalassaemia. Parent to child transmission route was only 21 (6%) (Table 2).

The district had four sub-divisions named Tamluk, Haldia, Contai and Egra. District head quarter was situated at Tamluk. Maximum 47% (156) of PLHA cases were from the Tamluk Sub-division followed by Egra 19% (62), Haldia 17% (57) and Contai 17% (56). Migratory people were more in the district due to presence of large industries like Haldia Petro-chemical, Kolaghat Thermal Power Plant and attractive sea beach at Digha (Figure 1).

Maximum cases were from rural areas of the district such as Panskura-I, II, Tamluk, Sutahata, Nanda Kumar and Bhagwanpur-II blocks.

DISCUSSION

Almost no gender discrimination found among the PLHAs in Purba Medinipur district. But most of the PLHAs were less educated and illiterate. Most of them were young, adult and their income of the family was also stumpy. Highest number of the PLHAs became infected through heterosexual route and their profession was laborer (and home maker in case of women). It was observed that maximum PLHAs was found in the age group of 15 – 49 years (85%) which was close to Indian scenario 89% [7]. There was no gender difference among the PLHAs but in overall National finding men with PLHAs were more than women.[7]

Due to uneven development of rural areas, people had to move for work at different industrial areas. Some of them were skilled labor and others were unskilled. They could not afford their family to live with them. They had a chance of sexual encounter with unknown people and due to illiteracy and ignorance as well as poverty they fail to practice safe sex and they acquired HIV infection.[8]

There was significant difference between urban and rural distribution of PLHAs. Most of the PLHA cases were from the rural areas though the concentration of PLHAs was higher in the proximity to the district head quarter. This finding was similar with the finding of National AIDS Control Organization (NACO).[9] But a technical report revealed that there was no difference of PLHAs distribution between urban and rural area of India.[10]

Children became infected by the parents usually. But a few PLHAs child acquired infection during blood transfusion for thalassaemia. It was observed, most PLHAs were found in 15 – 45 years (84%) which was close to Indian scenario 88.7%.[11] Heterosexual route of HIV transmission was found prevalent among the PLHAs. So it is important to counsel people about safe sex practice, awareness generation of HIV, its mode of transmission and prevention. It is necessary to improve social security, quality of life and opportunity of employment to the people to halt HIV spread.

REFERENCES

1. UNAIDS, WHO: AIDS Epidemic data, (unaids.org / pub / report / 2009 / jc1700_epi_updatee_en.pdf), Update, Dec. 2009.

2. World HIV/AIDS Statistics. Global HIV and AIDS estimates, 2009 and 2010 (www.avert.org/worldstat.htm)

3. UNAIDS, World AIDS Day Report 2011 (http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/JC2216_WorldAIDSday_report_2011_en.pdf)

4. National AIDS Control Organization, Department of AIDS Control; Ministry of Health & Family Welfare, Government of India: Annual Report 2010-11, (http://www.nacoonline.org/upload/REPORTS/NACO%20Annual%20Report%202010-11.pdf)

5. National AIDS Control Organization, Ministry of Health and Family Welfare; Government of India, A Technical Brief HIV Sentinel Surveillance and HIV Estimation 2007October 2008, (http://www.nacoonline.org/upload/Publication/M&E%20Surveillance,%20Research/HIV%20Sentinel%20Surveillance%20and%20HIV%20Estimation%202007_A%20Technical%20Brief.pdf)

6. Census of India 2001. (http://www.censusindia.gov.in/2001-common/ censusdataonline.html)

7. National AIDS Control Organization, Department of AIDS Control; Ministry of Health and Family Welfare, Government of India, HIV Data. (www.nacoonline.org/Quick_Link/HIV_Data).

8. Ranjan Gupta. HIV/AIDS and the future of the poor, illiterate and marginalized population, Theoritical Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA; ranjan@lanl.gov

9. National AIDS Control Organization, Ministry of Health & Family Welfare, Government of India, New Delhi, August 2009

10. The Technical Report: India HIV Estimate 2006, National AIDS Control Organization (NACO) and National Institute of Medical Statistics (NIMS).

11. HIV Sentinel Surveillance and HIV Estimation in India 2007, A Technical Brief, NACO, Ministry of H&FW, Govt. India, October 2008.

EVALUATION OF NITRATE REDUCTASE ASSAY FOR RAPID DETECTION OF DRUG RESISTANT TUBERCULOSIS

Sah RK[1], Bhatta DR[1], Ghimire GR[2], Kandel BP[3], Tiwari BR[4], Sherchand JB[5]

[1] Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2] National Tuberculosis Center, Thimi, Bhaktapur, Nepal
[3] Janapriya Multiple Campus, Pokhara, Nepal
[4] School of Health and Allied Sciences, Pokhara University, Pokhara, Nepal
[3] Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal

ABSTRACT

Introduction: Emergence of multidrug-resistant tuberculosis (MDR-TB) urgently demands for simple, rapid and inexpensive methods of its detection for the effective treatment of drug resistant tuberculosis, particularly in low-income countries.

Methodology: This prospective study was carried out at National Tuberculosis Reference Laboratory and South Asian Association of Regional Cooperation (SAARC) Tuberculosis and HIV/AIDS Centre, Thimi, Bhaktapur, Nepal, from November 2009 to May 2010 to evaluate nitrate reductase assay (NRA) efficacy for rapid determination of streptomycin, isoniazid, rifampicin and ethambutol susceptibility of *Mycobacterium tuberculosis* strains.

Results: A total of 113 clinical isolates of *M. tuberculosis* were tested for four first line antitubercular drugs by nitrate reductase assay and were compared with standard proportion method. Results were available in 7-14 days by NRA as compared to proportion method which generally takes 4-6 weeks. The sensitivity and specificity of NRA were 98.1% and 100% for isoniazid, 95.1% and 98.6% for rifampicin, 91.4% and 94.9% for streptomycin, and 78.6% and 97.9% for ethambutol, respectively. Agreement between NRA and proportion method were 99.1%, 97.3%, 93.8%, 95.6% for isoniazid, rifampicin, streptomycin and ethambutol, respectively.

Conclusion: NRA is easier, inexpensive and reliable method for susceptibility testing of *Mycobacterum tuberculosis* for isoniazid and rifampicin, the two most important drugs for the treatment of tuberculosis. The reduction in susceptibility testing time, and higher sensitivity and specificity of NRA method is of fundamental importance in detecting MDR-TB.

Key words: Drug Susceptibility, MDR-TB, NRA, Proportion Method

INTRODUCTION

Emergence of multidrug resistant tuberculosis (MDR-TB) during the past ten years in higher rate represents a major public health problem, especially in low-resource countries where the burden of the disease is higher. MDR-TB constitutes a serious threat for the effective control of the disease stressing the need for the rapid detection of drug resistance.[1] Conventional methods like proportion method (PM), the resistance ratio method and the absolute concentration method to detect drug resistance in *Mycobacterium tuberculosis* have traditionally relied on slow and cumbersome procedures requiring a minimum of 3–4 weeks to produce results.[2] Other methods, such as the BACTEC 460 TB System[3], and oxidation-reduction dyes, e.g. tetrazolium[4], and Microplate Alamar Blue Assay (MABA)[5], are faster but have the drawback of requiring either radioactive or expensive substrates, and are consequently not feasible in

Correspondence:
Ranjit Kumar Sah,
Central Department of Microbiology,
Tribhuvan University, Kirtipur, Nepal
Email: ranjitkmrsah@gmail.com

most resource-poor settings. A cost effective and rapid drug susceptibility method is required to guide TB treatment.

With similar characteristics to colorimetric methods, the Nitrate Reductase Assay (NRA) has been described, which is based on the ability of *M. tuberculosis* to reduce nitrate to nitrite after growth in the presence or absence of antibiotics. The use of specific reagents produces a change of color in the presence of nitrites indicating a positive result.[6] Since it is simpler, cheaper and rapid, evaluation of NRA as an alternative method for determining *M. tuberculosis* susceptibility to rifampicin (RIF), isoniazid (INH), streptomycin (STR) and ethambutol (EMB) is necessary.

METHODOLOGY

The NRA was performed on a total of 113 isolates of *M. tuberculosis*. Eighty three isolates were from cases reported for pulmonary TB at National Tuberculosis Center (NTC), Thimi, Bhaktapur, Nepal, between November 2009 and May 2010; and thirty isolates were of proficiency testing strain from Supranational Reference Laboratory, Germany.

The PM was carried out on Lowenstein and Jenson (LJ) medium according to the standard procedures with the recommended critical concentrations of 40µg/ml for rifampicin, 0.2µg/ml for isoniazid, 2µg/ml for ethambutol and 4µg/ml for streptomycin.[2] NRA was performed according to the already described protocol.[6] The following critical concentrations were used: 0.2µg/ml for INH, 40 µg/ml for RIF, 4µg/ml for STR and 2.0µg/ml for EMB. Briefly, fresh subculture (1µl loops of bacteria) from isolates of *M. tuberculosis* grown on LJ medium was taken and vortexed in small amount of sterile distilled water and turbidity was adjusted according to McFarland standard no.1. Part of the suspension was diluted 1:10 in sterile distilled water. For each isolate, 0.2 ml of suspension was inoculated into the tubes containing LJ medium with potassium nitrate (KNO_3) and the antitubercular drugs; 0.2 ml of the 1:10 dilution was inoculated into drug free media (LJ media) containing KNO_3 which served as growth controls. Tubes in triplicate were incubated at 37°C for 14 days and 0.5 ml of a mixture of three reagents (25µl of concentrated HCl, 50µl of 2% sulphanilamide and 50µl of 1% n-1-napthyl-ethylenediamine dihydrochloride) was added to one drug-free control tube after 7 days of incubation. If its colour changed to pink then tubes with drugs were tested. An isolate was considered resistant if there was colour change (pink or deep red to violet) in the drug tube in question greater than in the 1:10 diluted growth control on the same day. If the tubes did not show any colour change and remains the same, these were further incubated for 10 days and for 14 days as described.[6] Statistical analysis of data was carried out using χ^2 test at 5% level of significance using Statistical Package for Social Science (SPSS version 17.0).

RESULTS

Altogether 113 *M. tuberculosis* isolates were tested and the results were available in 7 days for 63.3% of the strains, in 10 days for 82.3% of the strains, and in 14 days for 100% of the strains by NRA method (Table 1).

Table 1. Number of days required for result by NRA method

No. of days	No. of specimens reported	Cumulative %
7	56	63.3
10	37	82.3
14	20	100.0

Of the total isolates, isolates showing resistance to INH, RIF, STR and EMB were 46.0%, 36.3%, 30.9% and 12.4% respectively, by the proportion method while resistance to respective drugs was 45.1%, 35.4%, 31.8% and 11.5% by NRA. The sensitivity and specificity for NRA were 98.1% and 100.0%, 95.1% and 98.6%, 91.4% and 94.9%, and 78.6% and 97.9% for INH, RIF, STR and EMB respectively (Table 2).

The results showed that NRA and proportion method do not differ significantly (P>0.05 for all the drugs). There was high agreement between both methods (Table 3) when tested against INH, RMP, SM and EMB with kappa, k=0.98, 0.93, 0.86 and 0.81 respectively.

Table 2. Comparison of Indirect Nitrate Reductase Assay results with Conventional Proportion Method

Drugs	Conventional Proportion method	Nitrate reductase assay method					
		Resistant	Sensitive	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)
INH	Resistant=52	51	1	98.1	100	100	98.4
	Sensitive=61	0	61				
RFP	Resistant=41	39	2	95.1	98.6	97.5	97.3
	Sensitive=72	1	71				
SM	Resistant=35	32	3	91.4	94.9	88.9	96.1
	Sensitive=78	4	74				
EMB	Resistant=14	11	3	78.6	97.9	84.6	97
	Sensitive=99	2	97				

Table 3. Percentage agreement between the Proportion and the NRA methods for susceptibility testing of *M. tuberculosis* to each drug tested

S.N.	Drugs	No. of isolates with the following results		Percent agreement
		PR method-Susceptible NRA method-Susceptible	PR method-Resistant NRA method-Resistant	
1	INH	61	51	99.1
2	RFP	71	39	97.3
3	SM	74	32	93.8
4	EMB	97	11	95.6

DISCUSSION

Traditional drug susceptibility testing such as the PM on LJ or agar medium is time consuming. For developing countries, it would be helpful to have a simple and inexpensive test that can rapidly detect resistant *M. tuberculosis* strains. The reporting time of NRA was between 7-14 days with majority in 10 days as against 28-42 days for the conventional PM. This is very much comparable with the results of MGIT and BACTEC 460TB, which require the use of expensive instruments and high running cost. The rapidity with which test could be performed has also been reported with comparable accuracy to that of NRA such as the MTT or resazurin assays.[7] However, they make use of liquid medium in a micro-plate format and that makes the technique more complex and might also constitute a biohazard. Instead, the NRA utilizes standard solid LJ-medium, although with KNO_3 incorporated and it could therefore be easily adopted in any culture laboratory.

Nitrate reductase-negative strains of *M. tuberculosis* are very unusual[8] and on the other hand, false susceptible results would in this case be detected by the lack of a positive reaction also in drug free growth. *M. bovis* does not reduce nitrate, therefore the NRA technique is not applicable. Disadvantages of NRA are; the culture is killed by the mix reagent used to develop the assay, requiring that multiple cultures be prepared if comparative testing will be performed and only fresh cultures must be used (<14 days).

In present study, a high level of agreement between NRA and PM was determined for isoniazid and rifampicin. However, despite a higher agreement between NRA and PM for streptomycin (93.8%) and ethambutol (95.6%), low sensitivity of streptomycin (91.4%) and ethambutol (78.6%) was found. For isoniazid and rifampicin, sensitivity and specificity was found to be high; 98.1% and 100.0%, and 95.1% and 98.6% respectively. These results are very important since rifampicin and isoniazid are the two most important drugs used in the treatment of TB. [9] Higher accuracy results for INH, RIF and EMB and lower accuracy results for STR were shown in a similar multicenter study. [10] The percentage agreement of 98.3%, 98.3%, 90.8% and 93.3% by NRA for INH, RMP, SM and EMB respectively, were observed in Brazilian study. [11] However, marginally lower sensitivity and specificity for STR and EMB have also been

reported. [12, 7] For STR and EMB testing, further studies are required to optimize preparation of adequate inoculum and drug concentration in order to avoid strain misclassification.

CONCLUSION

In the context of emergence of MDR-TB, the NRA may be of great importance due to its higher sensitivity and specificity for the rapid detection of rifampicin and isoniazid resistance, the two most important drugs for tuberculosis treatment.

ACKNOWLEDGEMENTS

The authors acknowledge all the laboratory staffs of NTC and SAARC TB/HIV Center, for their worthy cooperation during the study.

REFERENCES

1. Caminero JA. Multidrug-resistant tuberculosis: epidemiology, risk factors and case finding. *Int J Tuberc Lung Dis*. 2010;14:382-90.

2. Canetti G, Fox W, Khomenko A, Mahler HT, et al. Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Org. 1969;41:21-43.

3. Roberts GD, Goodman NL, Heifets L, et al. Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of Mycobacterium tuberculosis from acid-fast smear-positive specimens. *J Clin Microbiol*. 1983;18:689-96.

4. Caviedes L, Delgado J, Gilman RH. Tetrazolium microplate assay as a rapid and inexpensive colorimetric method for determination of antibiotic susceptibility of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2002;40:1873-74.

5. Franzblau SG, Witzig RS, McLaughlin JC, et al. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar Blue assay. *J Clin Microbiol*. 1998;36:362-66.

6. Angeby KA, Klintz L, Hoffner SE. Rapid and inexpensive drug susceptibility testing of *Mycobacterium tuberculosis* with a nitrate reductase assay. *J Clin Microbiol*. 2002;40:553-55.

7. Montoro E, Lemus D, Echemendia M, Martin A, Portaels F, Palomino JC. Comparative evaluation of the nitrate reduction assay, the MTT test, and the resazurin microtitre assay for drug susceptibility testing of clinical isolates of *Mycobacterium tuberculosis*. *J Antimicrob Chemother*. 2005;55:500-5.

8. Kent PT, Kubica GP. Pubic health mycobacteriology: A guide for the level III laboratory. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention. Atlanta, Georgia, USA. 1985:159-84.

9. World Health Organization (WHO). The WHO/ International Union Against Tuberculosis and Lung Disease. Global Project on Anti-tuberculosis Drug Resistance Surveillance. Antituberculosis Drug Resistance in the World, report no. 3. World Health Organization. Geneva, Switzerland. 2004.

10. Martin A, Montoro E, Lemus D, et al. Multicenter evaluation of the nitrate reductase assay for drug resistance detection of *Mycobacterium tuberculosis*. *J Microbiol Methods*. 2005;63:145-50.

11. Shikama Mde L, Silva RR, Martins MC, et al. Rapid detection of resistant tuberculosis by nitrate reductase assay performed in three settings in Brazil. *J Antimicrob Chemother*. 2009;64:794-96.

12. Lemus D, Montoro E, Echemendia M, Martin A, Portaels F, Palomino JC. Nitrate reductase assay for detection of drug resistance in *Mycobacterium tuberculosis*: simple and inexpensive method for low-resource laboratories. *J Med Microbiol*. 2006;55:861-63.

KNOWLEDGE REGARDING HIV/AIDS AMONG PREGNANT FEMALES ATTENDING ANTENATAL CLINIC AT KHYBER TEACHING HOSPITAL PESHAWAR, PAKISTAN

Fatima S[1], Afridi NK[2], Khan S[3]

[1] Khyber Teaching Hospital, Peshawar, Pakistan
[2] SAARC TB & HIV/AIDS Centre, Kathmandu, Nepal
[3] Federal Medical Centre, Peshawar, Pakistan

ABSTRACT

Introduction: Globally, around 34 million people are living with HIV in 2010, and 35% of the pregnant women are tested for HIV in the low and middle income countries. HIV infection in pregnancy has become a complication of pregnancy in some developing countries. This has major implications for the management of pregnancy and birth. With an estimated one and a half million HIV-positive women becoming pregnant each year, almost 600,000 children will be infected by mother-to-child transmission annually.

Methodology: A cross sectional study was conducted from October – December 2011 in the Department of Obstetrics and Gynecology, Khyber Teaching Hospital, Peshawar. It is a tertiary care hospital located in Peshawar. A sample of 200 pregnant females was used to accomplish the study. The targeted study population was all antenatal attendees who were visiting the hospital within the current pregnancy. A pre-coded, pre- tested structured questionnaire was used to gather information on the study variables. The questionnaire included variables related socio-demographics e.g., age, education status, occupation, residence. It also included the gestational age and gravidity of the pregnant females.

Results: A total of 200 pregnant females attending the antenatal clinic participated in the study. The age range of the respondents was 17 to 45 years. Age range 17-25 years accounted for the highest percentage (52%). Around 70% of the respondents had no formal schooling while only 18% had got secondary education. Ninety – seven percent of the females were housewives while only 3% were employed. Out of the total respondents (200) only 86 (43%) have heard of HIV while 114 (57%) respondents have not heard of HIV.

Conclusion: The study concluded that only 43% of the pregnant females have heard of HIV. Although this is not promising still there is room for improvement. Health education targeting pregnant females is essential.

INTRODUCTION

Globally, around 34 million people are living with HIV in 2010, and 35% of the pregnant women are tested for HIV in the low and middle income countries.[1] The United Nations General Assembly Special Session (UNGASS), in 2001, put a clear emphasis on the effect of HIV/AIDS on maternal and child health. The final declaration of commitment from the assembly stated that the proportion of infants infected with HIV should be reduced by 20% by 2005, and by 50% by 2010.[2]

HIV infection in pregnancy has become a complication of pregnancy in some developing countries. This has major implications for the management of pregnancy and birth. With an estimated one and a half million HIV-positive women becoming pregnant each year, almost 600,

Correspondence:
Dr. Shireen Fatima
Khyber Teaching Hospital
Peshawar, Pakistan
E-mail: shireenfatima877@yahoo.com

000 children will be infected by mother-to-child transmission annually.[3]

HIV is transmitted mainly in three ways: through unprotected sexual intercourse, heterosexual or homosexual; through blood or blood products, donated semen or organs; or from an infected mother to her child (vertical or mother-to-child transmission). More than 70% of infections are a result of heterosexual transmission and over 90% of infections in children result from mother-to-child transmission.[4]

Data available from developed countries suggest that pregnancy does not accelerate the progression of HIV disease. A systematic review and meta-analysis of seven cohort studies from 1983 to 1996 suggested that there was an association between adverse maternal outcomes and pregnancy in HIV-infected women. Routine antenatal counseling and testing for HIV, also known as provider-initiated testing approach, involves testing all antenatal attendees for HIV, apart from those who decline the test (i.e., those who opt out). This is the standard of care in Scandinavia and other high-income countries.[5,.6] The sub-Saharan countries, in a bid to increase HIV testing rates, routine antenatal HIV counseling and testing was successfully introduced in the HIV prevention programmes of several countries in line with the Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) recommendations.[7]

Wherever possible, voluntary counseling and testing should be available to any pregnant women who request it and offered to all in areas of moderate or high prevalence. Routine testing of pregnant women without consent or without access to counseling is, however, an unacceptable practice and the disadvantages may negate any benefit obtained from knowing the HIV status of the women. These include a reluctance to utilize maternity services through fear of discrimination, denial of a positive diagnosis and stigmatization. Recommendations for mandatory testing of pregnant women or newborns have led to concern about the autonomy and rights of women.[8,9] Complications of early pregnancy have been associated with HIV infection in several studies.[10,11] HIV-1 and HIV-2 infection in Africa have both been linked to a higher rate of spontaneous abortion. HIV sero-positive women were 1.47 times more likely to have had a previous spontaneous abortion and this rose to 1.81 in women in Uganda who were sero-positive for both HIV and syphilis.[12]

In many developed countries, HIV testing, antiretroviral therapy and infant feeding modifications have been used to virtually eliminate mother-to-child transmission of HIV yet Sub-Saharan Africa continues to be heavily affected due to lack of knowledge, testing services and antiretroviral therapy.[13]

It has been observed that most of the individuals in community do not have correct and complete information about HIV/ AIDS and its prevention. And many of the female population are unaware about correct knowledge of HIV/AIDS. The study was conducted with the aim to determine the knowledge of pregnant females regarding HIV/ AIDS attending a tertiary care hospital in Peshawar.

METHODOLOGY

A cross sectional study was conducted from October – December 2011 in the Department of Obstetrics and Gynecology, Khyber Teaching Hospital, Peshawar. It is a tertiary care hospital located in Peshawar. A sample of 200 pregnant females was used to accomplish the study. The sample size was calculated by assuming that knowledge level of the females on HIV/AIDS being 20% and the maximum acceptable difference from true value is.06, then for a significance value of 5%, the sample size came out to be 171. To account for non-response the sample size was inflated by 15% to give the sample size of 197. The final sample size was 200.

The targeted study population was all antenatal attendees who were visiting the hospital within the current pregnancy. Women, who were very sick, requiring urgent medical attention, were excluded from the study. Consent was taken from each of the women before starting the interview.

A pre- coded, pre- tested structured questionnaire

was used to gather information on the study variables. The questionnaire included variables related socio-demographics e.g., age, education status, occupation, residence. It also included the gestational age and gravidity of the pregnant females. The variables related to knowledge, attitude and practices were whether heard of HIV, source of information, whether HIV transmitted by kissing, sharing food, mosquito bite, through contaminated needles, blood products, breast milk etc. The interviews were conducted in local language. The principal investigator checked filled questionnaires for completeness at the end of each day.

Data was entered and analyzed in SPSS version 16.0. Descriptive analysis of frequencies and percentages were generated for the variables. The association between different variables was determined using chi square test and the significance level was set at 0.05.

RESULTS

A total of 200 pregnant females attending the antenatal clinic participated in the study. The age range of the respondents was 17 to 45 years. Age range 17-25 years accounted for the highest percentage (52%) while those 36 - 45 years of age group accounted for 5% (Figure 1). Around 70% of the respondents had no formal schooling while only 18% had got secondary education. Ninety – seven percent of the females were housewives while only 3% were employed.

It was observed that majority of the antenatal women 142(71%) were registered for antenatal check up in the third trimester while only 18 (09%) came for antenatal registration / check up in the first trimester. The participants who were first, second to fourth and more than four gravida were 32%, 39% and 29% respectively.

Out of the total respondents (200) only 86 (43%) have heard of HIV while 114 (57%) respondents have not heard of HIV (Figure 2).The rest of the questionnaire was administered only to those participants who have heard of HIV while the participants who have not heard of HIV were given a brief introduction of HIV /A IDS and its prevention. Regarding source of information of HIV around 74% of the participants reported that the main source of information was mass media followed by friend/relative (21%) as reported by the participants (Figure 3). Table 1 summarizes the knowledge regarding HIV of pregnant women who have heard of HIV. Although most of the responses given by the participants were correct yet a few misconceptions still exists as evident by the responses. Around 44% of the participants responded that HIV can be transmitted by kissing/ hugging, 36% believed that clothes can act as an agent in the transmission of HIV while 32% responded that sharing/ eating food can transmit HIV. A higher percentage of participants responded that HIV transmission can occur due to sex with person having HIV (84%), contaminated needle/syringe (87%), contaminated blood transfusion (92%) and HIV infected mother to child transmission (70%).

Table 2 depicts association between two variables i.e., heard of HIV and age category, it shows that the association between the two variables are not significant p -value (0.54) so different age categories have no association with the variable heard of HIV.

Table 3 depicts the association between two variables i.e., heard of HIV and education status categories. And it was found the two variables are highly significant p-value (0.000), it was also evident that around 71% of the women who have not heard of HIV have no formal schooling. It is also interesting to note as the education level becomes higher then more females are educated and have heard of HIV.

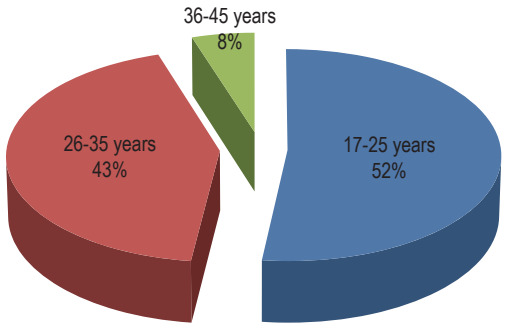


Figure 1. Age distribution of the pregnant females

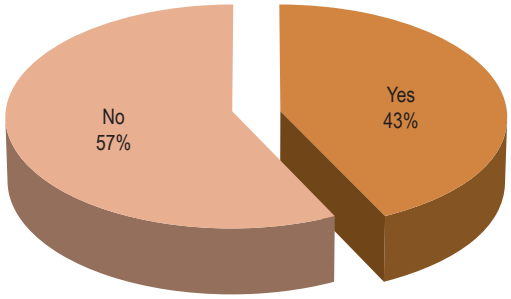


Figure 2. Knowledge regarding HIV

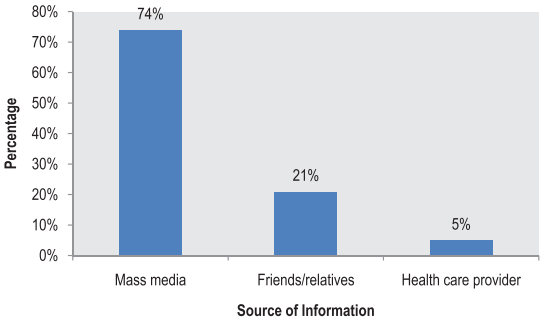


Figure 3. Source of Information regarding HIV

Table 1. Frequency analysis about knowledge regarding HIV (n = 86)

Variables	Number (%)
HIV transmitted by kissing/hugging	
Yes	38 (44)
No	48 (56)
HIV transmitted by clothes	
Yes	31(36)
No	55 (64)
HIV transmitted by eating food with HIV person	
Yes	28 (32)
No	58 (68)
HIV transmitted by having sex with HIV person	
Yes	72 (84)
No	14 (16)
HIV transmitted by bite of mosquito	
Yes	58 (67)
No	28 (33)
HIV transmitted by contaminated needle/syringe	
Yes	75 (87)
No	11(13)
HIV transmitted by contaminated blood transfusion	
Yes	79 (92)
No	07 (08)
HIV transmitted by HIV mother to child	
Yes	60 (70)
No	26 (30)
HIV transmitted through breast milk	
Yes	48 (56)
No	38 (44)
Condom prevent HIV transmission	
Yes	35 (41)
No	51 (59)
AIDS curable disease	
Yes	40 (46)
No	46 (54)

Table 2. Cross Tabulation between Age group and heard about HIV (n = 200)

	Heard of HIV	
Age Group	Yes	No
17 - 25 years	41 (40%)	62 (60%)
26 - 35 years	39 (45%)	47 (55%)
36 - 45 years	6 (55%)	5 (45%)

χ^2: 1.22
p-value: 0.54

Table 3. Cross Tabulation between Education status and heard about HIV (n = 200)

Education category	Heard of HIV	
	Yes	No
No formal schooling	41 (29%)	98 (71%)
Primary schooling	06 (60%)	04 (40%)
Secondary schooling	25 (69%)	11 (31%)
Above Secondary schooling	14 (93%)	01 (07%)

χ^2: 1.22
p-value: 0.000

DISCUSSION

Knowledge of HIV/AIDS among pregnant females was not appreciably high as 57% have not heard of HIV/AIDS. Only 43% of the pregnant females have heard of HIV. These findings are also shown in a study conducted by Singh where about 40% of pregnant women had heard of AIDS.[14] One of the most important finding in this study was that education levels play a significant role in awareness regarding HIV/AIDS. So the higher educational status of pregnant women was associated with the increase in awareness towards HIV/AID. Similar findings are reported by a number of studies conducted.[15, 16]

The main source of information regarding HIV/AIDS was mass media (74%) and many studies

have corroborated this finding. The significance of information, education and communication in prevention and control of HIV/AIDS had been advocated.[17]

Misconceptions regarding HIV/AIDS are still prevalent as 44% of the participants believed that HIV is transmitted by kissing/hugging, 36% responded that it is spread by wearing clothes of infected person. There was also misconception about eating food with HIV infected person and mosquitoes' bite can transmit HIV infection. The issue of misconceptions was also highlighted in various studies. [18, 19] Lack of precise and correct information and knowledge about sexual health has resulted in myths and misconceptions about sex and HIV, contributing to increasing transmission rates as well as stigma and discrimination towards people living with HIV/AIDS.

Approximately 50% of the pregnant females belong to 17 – 25 years age group and giving them health at this stage might help them in preventing themselves and their newborns from the risk of HIV/AIDS. A study conducted in Pakistan also showed that 58.7% of the participants responded that use of condom can prevent the transmission of HIV/AIDS while in this study 41% responded that condom can prevent HIV.[20]

Whereas breastfeeding carries significant health benefits to infants and young children, HIV can

be transmitted during breastfeeding from an HIV-infected mother to her infant. Reducing this

transmission while ensuring improved HIV-free survival is one of the most pressing public health dilemmas confronting researchers, health-care professionals, health policy-makers and HIV-infected women in many areas of the world, especially in developing countries. Mother-to-child transmission of HIV can occur during pregnancy, labour or delivery, or through breastfeeding. Without specific interventions, HIV-infected women will pass the virus to their infants during pregnancy or delivery in about 15-25% of cases; and an additional 5-20% of infants may become infected postnatally during breastfeeding, for an overall risk of 30-45%.

Breastfeeding may thus be responsible for one third to one half of HIV infections in infants when interventions are not available.[21]

Areas of concern include the low level of knowledge gained directly from health care workers and only 04 (05%) of the pregnant females responded that they have heard HIV from health care workers. There is a perturbing lack of knowledge about the breastfeeding routes of transmission particularly as the participants were pregnant women, as 56% responded that HIV can be transmitted through breast milk. Awareness that condoms can prevent HIV transmission was relatively low 41% and also that 46% responded that AIDS is a curable disease is also alarming. These areas should form part of the health education imparted during each antenatal session and should be supported with the use of audio-visual aids.

Although the scientific community are struggling to find a long lasting cure for HIV/AIDS, till now there is no effective treatment available nor has a vaccine breakthrough been made for HIV/AIDS. Advocacy, general awareness, heath education, safe sex practices, screening of blood products are the best recommended practices.

CONCLUSION

The study concluded that only 43% of the pregnant females have heard of HIV. Although this is not promising still there is room for improvement. Health education targeting pregnant females is essential. Awareness campaigns regarding HIV/AIDS involving mass media and through health care providers can be helpful and beneficial in prevention and spread of HIV/AIDS.

REFERENCES

1. World Global HIV/AIDS response, Progress Report, World Health Organization, 2011.
2. United Nations General Assembly. Final declaration of commitment on HIV/AIDS, New York: 2001 http://www.Unaids.Org/UNGASS/index.html.
3. UNAIDS. Report on the Global HIV/AIDS epidemic. Geneva, Joint United Nations Programme on HIV/AIDS, 1997:1-13.

4. Fowler MG, Melnick SL, Mathieson BJ. Women and HIV. Epidemiology and global overview. Obstet Gynecol Clin North Am, 1997;24:705-29.

5. CDC: Revised Recommendations for HIV Screening of Pregnant Women. In MMWR Recomm Rep. Volume 50. Atlanta: CDC; 2001;59-86 [http://www. cdc.gov/mmwr/preview/mmwrhtml/rr5019a2.htm].

6. Sherr L, Fox Z, Lipton M, Whyte P, Jones P, Harrison U, Group CIS: Sustaining HIV testing in pregnancy - Evaluation of routine offer of HIV testing in three London hospitals over 2 years. AIDS Care: Psychological and Socio-medical Aspects of AIDS/HIV 2006; 18:183-88.

7. UNAIDS/WHO: UNAIDS/WHO policy statement on HIV testing Geneva; 2004 [http:// www.who.int / hiv / pub / vct / statement / en/index.html].

8. Minkoff H, Willoughby A. The future of prenatal HIV testing. Acta Pediatr, 1997;421:72-77

9. Minkoff H, Willoughby A. Pediatric HIV disease, zidovudine in pregnancy and unblinding heelstick surveys. JAMA, 1995;274:1165-68.

10. D'Ubaldo C, Pezzotti P, Rezza G et al. Association between HIV-1 infection and miscarriage: a retrospective study. AIDS, 1998;12:1087-93.

11. Langston C, Lewis DE, Hammill HA et al. Excess intrauterine fetal demise associated with maternal human immunodeficiency virus infection. J Infect Dis, 1995;172:1451-60.

12. Byabamazina CR, Asiimwe OG, Malamba S et al. HIV/Syphilis serology as an indicator of past pregnancy outcomes among antenatal attendees in Kampala. IXth International Conference on AIDS and STD in Africa, Kampala, 1995, Abstract TuC108.

13. US Aids for International Development (USAID). Nigeria a Country profile (HIV/AIDS); 2003 www. usaid.gov / acations / sub_sahara africa/countries/nigeria.

14. Singh S, Fukuda H, Ingle Gk, Tatara K. Knowledge, attitude, the perceived risks of infection and sources of information about HIV/AIDS among pregnant women in an urban population of Delhi. J Commun Dis 2002;34:23-34.

15. Ambati BK, Ambati J and Rao AM. Dynamics of knowledge and attitudes about AIDS among the educated in Southern India. AIDS Care 1997;9:319-30.

16. Jacob KS, Jayakumari H, Jacob K John, Jacob John T. Awareness of AIDS in India: effect of public education through the mass media. BMJ 1989;229:721.

17. Femi Oyewo MN. Information, Education and Comunication in the Prevention and Control of HIV/AIDS. West Afr. J.Pharm. 2003;1:25-32.

18. Ojieabu WA, Erah PO, Okafor NA. HIV/AIDS knowledge and sexual behaviour. Int J Health Res. March 2008;1:35

19. Bhattacharya GC, Cleland, and Holland. Knowledge about HIV/AIDS, the perceived risks of infection and sources of information of Asian-Indian born in the USA. AIDS Care 2000;2:203-09.

20. Gulfareen Haider, Nishat Zohra, Nusrat Nisar, Aftab Afroz Munir . Knowledge about AIDS/HIV infection among women attending obstetrics and gynaecology clinic at a university hospital. JPMA 2009;59:95

21. World Health Organization, 2008. HIV transmission through breastfeeding: a review of available evidence - an update from 2001 to 2007.

TUBERCULOSIS OR MELIOIDOSIS? - LOOK TWICE IN SOUTHWESTERN COASTAL INDIA

Mukhopadhyay C[1], Krishna S[2], KE Vandana[3], Saravu K[4], Balasubramaniam R[5]

[1,2] Kasturba Medical College, Manipal, India
[3,4,5] Kasturba Medical College Hospital, Manipal, India

ABSTRACT

Melioidosis is known endemic in many Southeast Asian countries, especially Thailand, and in Northern Australia. The disease was long considered under-recognized in India but has now gained the status of emerging infectious disease here. Increasing isolation of the causitive agent *B. pseudomallei* is encountered in the recent years from this part of western coastal India. We report one such case of pulmonary melioidosis in an elderly patient misdiagnosed as tuberculosis ending fatally. Prompt microbiological diagnosis prevents the unnecessary Anti-TB treatment or prophylaxis. This was also our first of isolation of *B. pseudomallei* from endotracheal aspirate.

Key words: Burkholderia pseudomallei, Melioidosis, Pneumonia, Tuberculosis

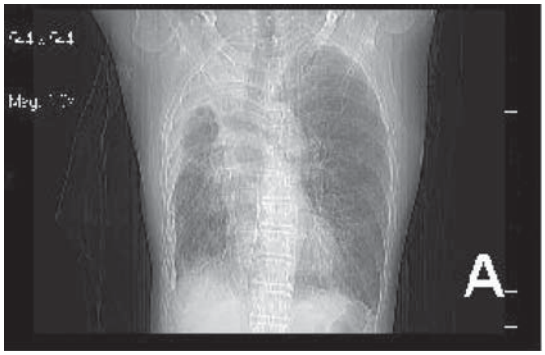
INTRODUCTION

Melioidosis is an endemic disease in Southeast Asia, especially in Thailand and also in Northern Australia. Humans acquire infection by exposure to *Burkholderia pseudomallei* present in soil and surface water.[1] Clinical presentations range from localized infections to fulminant septicaemia. Lung is the ommonest organ involved in nearly 48% of cases.[2.] Pulmonary melioidosis can present as mild undifferentiated pneumonia, which can be acute or subacute in nature to fulminant septic shock with high mortality. Subacute and chronic presentations mimic pulmonary tuberculosis clinically and radiologically making differentiation difficult.[3] There has been increasing reporting of cases from south-western coastal India, majority of which are from tertiary care centers.[4–6.] A combined effort of microbiologists in prompt identification of the bacilli, testing with the right antibiotics and a high index of suspicion from the clinicians leading to appropriate diagnosis and management is required in TB endemic areas such as India.[10]

Correspondence:
Dr. Sushma Krishna
Assistant Professor
Department of Microbiology
Amrit Institute of Medical Sciences
Cochin – 682 041, Kerala, India.
E-mail: sushmakrishna@aims.amrita.edu

Case report

An 80-year-old retired school teacher diagnosed with ischemic heart disease (IHD), hypertension and multi-infarct dementia for 2 years, on treatment, presented with sudden onset of fever and severe breathlessness for 3 days. At presentation, he was semi-conscious, cyanosed with bilateral crepitations all over the chest. He was provisionally diagnosed as a case of pulmonary tuberculosis 2 years ago with cough, breathlessness, elevated ESR and suggestive radiological findings on chest x-ray (Figure 1 A) and CT scan (Figure 1 B) though microbiologically there was no evidence of mycobacteria in sputum microscopy, culture and



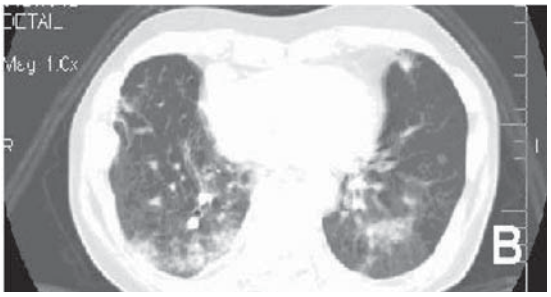


Figure 1 A, B: Chest X ray and CT scan in 2006 at the time of 1st presentation.

PCR. The patient had refused the planned anti-tubercular therapy. Further interrogation revealed that he had similar intermittent breathlessness with chest discomfort for last 4 years. He was non diabetic, did not smoke or consume alcohol. He was a bare foot walker and a snuff (a finely powdered tobacco used for inhalation by rural folk in India) abuser for 30 years.

During the current admission, the patient was ventilated with falling saturation. Investigations revealed elevated blood urea, creatinine and alkaline phosphatase levels. The chest X-ray showed air space opacities with radiolucent areas within the left paracardiac region suggestive of consolidation with breakdown signifying active disease (Figure 1 C). The unconfirmed working diagnosis at this stage included pneumonia with adult respiratory distress syndrome (ARDS) or reactivation of tuberculosis. He was empirically started on azithromycin and piperacillin-tazobactum along with other supportive therapy. However bilateral opacity in the serial chest X-ray (Figure1 D) showed progression and clinical condition worsened which were also thought to be result of ventilator associated pneumonia (VAP) and antimicrobial therapy was switched over to meropenem, teicoplanin and metronidazole on 5th day of ventilation. Meanwhile, the first 2 samples of ET aspirate had no growth after 48 hours; 3rd sample on 5th day of ventilation grew B. pseudomallei, sensitive to ceftazidime, piperacillin, co-trimoxazole, doxycyclin, meropenem, and resistant to amoxicillin-clavulanic acid, ciprofloxacin and gentamicin. The isolate had the typical safety-pin appearance on gram stain, and was biochemically identified and confirmed by API (biomeruix, France) as B. pseudomallei. Fungal culture of ET aspirate, AFB and blood culture were sterile. Although the patient showed a very brief clinical improvement with meropenem initially, he developed hypotension with falling platelets and rising serum creatinine levels despite aggressive therapy and succumbed to multi-organ dysfunction/ septic shock secondary to pulmonary melioidosis on the 13th day of hospitalization.

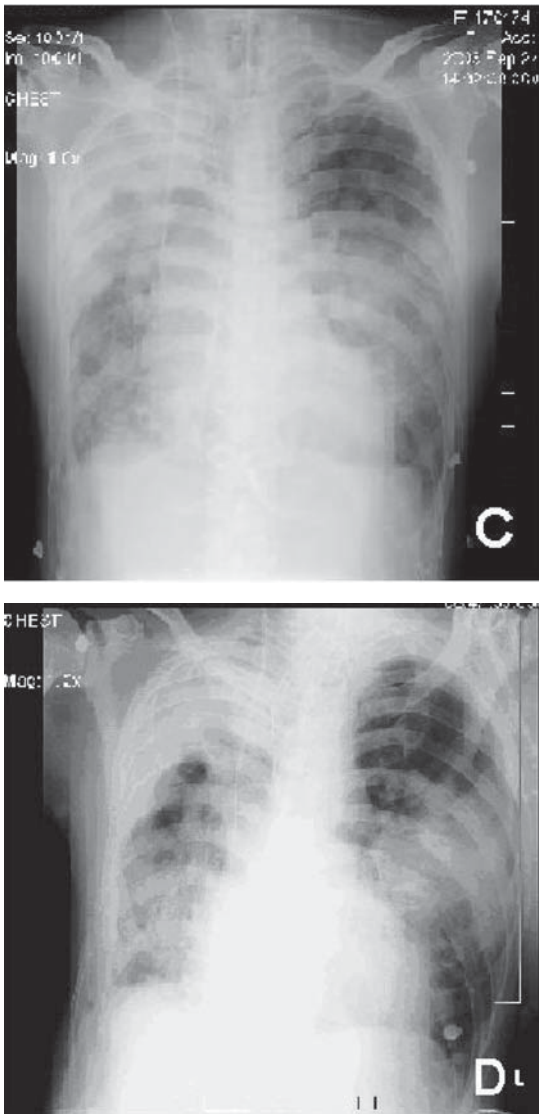


Figure 1 C, D: Chest X ray in 2008 at the time of last presentation at admission and day of expiry

DISCUSSION

Pulmonary melioidosis mimics tuberculosis both clinically and radiologically and is often misdiagnosed as tuberculosis, more so in endemic countries like India where both diseases coexist.[2] Patients with chronic pulmonary melioidosis also have fever, weight loss and productive cough, sometimes with haemoptysis like TB. Pleuritic

chest pain occurs in half of the patients.[8] Disease may often slowly progressive over months like TB. It can also be remitting and relapsing over many years, and acute deterioration with septicaemia may also occur**.** On chest radiography, they often have diffuse nodular infiltrates throughout both lungs, which coalesce, cavitate and progress rapidly, consistent with caseous necrosis and multiple metastatic abscess formation. Acute pneumonia with upper lobe consolidation in endemic regions warrants consideration of melioidosis**.**[3,8,9] The loss of volume on the right lung due to fibrothorax, pleural calcification and fibrocavitatory changes with consolidatory changes in right upper lobe and basal segments of lower lobes and areas of patchy opacity in bilateral posterior segments in CT scan (Figure 1 B) and on chest X-ray (Figure 1 A) support the earlier reports that pulmonary melioidosis can have varied radiological findings, which make it more difficult in arriving at the right diagnosis and often mistaken for TB.[3]

B. pseudomallai is a natural inhabitant of soil and water in tropics and subtropics. Inoculation through skin abrasions from contaminated soil, inhalation, aspiration are the various modes of transmission[7]. The mode of acquisition in this case might have been through inhalation or through an unnoticed inoculation attributed to bare foot walking for many years.

B. pseudomallai is isolated from wide range of specimens like blood, body fluids, sputum, pleural fluid, pus, wound swab in the laboratory, and our first time from ET aspirate.[9] This case marks out the disease as a true ticking 'Vietnamese time bomb' probably from many years where it followed a chronic course with a rapid fulminant outcome. Since the course of community-acquired pneumonia by *B.* pseudomallei is associated with high mortality rate, it becomes life saving to arrive at an accurate faster diagnosis. The co-morbid conditions of the patient like the advanced age, pre-existing hypertension, IHD and chronic obstructive pulmonary disease (COPD) collectively might have contributed to the fatal outcome.

The laboratory diagnosis of *B.pseudomallei* is not difficult if promptly looked for. It grows in 24-48hrs on the routine culture media used in laboratories like the Blood and Macconkey agar. Simple biochemical manual tests for non-fermenting gram-negative bacilli like a positive oxidase test, motility, polymixin –B resistance and dihydrolysis of arginine are sufficient for the lead and can be further confirmed by automated instruments if available. Molecular confirmation is not routinely necessary, more so in developing countries.

Therapy for Melioidosis too is also long term like TB. The initial intensive therapy consists of Ceftazidime, 50 mg/kg up to 2g, every 6 h or Meropenem, 25 mg/kg up to 1 g, every 8h or Imipenem, 25 mg/kg up to 1g, every 6h with or without Trimethoprim/sulfamethoxazole, 8/40mg/kg up to 320/1600 mg, every 12 h for a minimum of 14 days. The recommended eradication therapy includes Trimethoprim/sulfamethoxazole, 8/40 mg/kg up to 320/1600 mg, every 12 h with or without Doxycycline, 2 mg/kg up to 100 mg, 12 h for a minimum of 3 months. Accurate microbiological and clinical diagnosis is important in pulmonary melioidosis, which may be rapidly fatal in patients, especially with the existing pre-morbid conditions. Respiratory care practitioners may well be aware of the same that B.pseudomallei is always a potential pathogen and not to disregard it as a colonizer of the respiratory tract. TB in India many times stands over-diagnosed leading to unnecessary administration of Anti TB therapy. Rifampicin, an Anti-TB drug, is a known sterilizer and false clinical improvement in pulmonary melioidosis may sometimes be encountered. The burden, endemicity and public health importance of melioidosis may not be at present comparable to the magnanimity of TB for the formulation of national programs in our country for its diagnosis and prevention. Nevertheless, judicious judgment from the clinicians is required to look beyond TB in South Western Coastal India, an increasing place for isolation of *B*. pseudomallei.

ACKNOWLEDGMENT

Our special thanks to the deceased patient and his family, Indira Bairy, Professor, Department of Microbiology and Dr. Smithi, Dept of Radiology, Kasturba Medical College and Hospital, Manipal.

The publication of this case report has been approved by the relatives of the deceased patient.

REFERENCES

1. Dance DA. Ecology of *Burkholderia pseudomallei* and the interactions between environmental *Burkholderia spp*. and human-animal hosts. Acta Trop 2000;74:159-68.
2. Currie BJ. Melioidosis: an important cause of pneumonia in residents of and travellers returned from endemic regions. Eur Respir J 2003;22:542-50
3. Reechaipichitkul W. Clinical manifestations of pulmonary Melioidosis in adults. Southeast Asian J Trop Med Public Health 2004;35:664-9.
4. Chiranjay M, Kiran C, Sushma K, Nagalakshmi N, Sugandhi P Rao, Indira Bairy. Emergence of *Burkholderia Pseudomallei* and pandrug resistant non-fermenters from southern Karnataka, India. Transactions of the Royal Society of Tropical Medicine and Hygiene 2008;102:S12-S17
5. Vidyalakshmi K, Shrikala B, Bharathi B, Suchitra U. Melioidosis: an under-diagnosed entity in western coastal India: a clinico-microbiological analysis. Indian J Med Microbiol 2007;25:245- 8.
6. Vidyalakshmi K, Chakrapani M, Shrikala B et al. Tuberculosis mimicked by melioidosis. Int J Tuberc Lung Dis. 2008;12:1209-15.
7. Suputtamongkol Y, Hall AJ, Dance DAB et al. The epidemiology of melioidosis in Ubon Ratchatani, Northeast Thailand. Ind J Epidemiol 1994;23:1082-90.
8. Everett ED, Nelson RA. Pulmonary melioidosis: Observations in thirty-nine cases. Am Rev Respir Dis 1975;112:331-40.
9. Mary IP, Osterberg LG, Chau PY, Raffin TA. Pulmonary Melioidosis, Chest 1995;108;1420-4.
10. Chaowagul W, White NJ, Dance DAB et al. Melioidosis: a major cause of community acquired septicemia in Northeastern Thailand. J Infect Dis 1989;159:890-9.

PATIENT CHARACTERISTICS ASSOCIATED WITH NON-ADHERENCE TO ANTI TUBERCULOSIS TREATMENT IN SRI LANKA

KAS Jayawardena, C Sarukkali, AKSB de Alwis, S Samaraweera, D Waidyaratne, S Nawaratne, R Perera, Darmasiri R, A Kariyawasam, RASE Illangakoon, DNK Wijesingha, PM Karunadasa

National Programme for Tuberculosis Control and Chest Diseases, Sri Lanka

ABSTRACT

Introduction: Sri Lanka had achieved a significant improvement in the tuberculosis control including a reduction of the default rate since introduction of the DOTS strategy to the National TB control Programme in 1997. Patients' adherence to anti TB treatment may be measured using either process oriented or outcome oriented definitions. Default rate is an outcome oriented definition that may be used as an indicator of poor patient adherence to anti TB treatment. One of the main obstacles in achieving the best tuberculosis (TB) control is that patients do not complete full course of anti TB treatment. A prospective cohort study was carried out with the objective to identify patient characteristics that associate with defaulting anti tuberculosis treatment

Methodology: This was a prospective cohort study of new smear positive pulmonary tuberculosis patients registered for treatment between 1.6.2008 and 31.8.2008 in seven districts in Sri Lanka. Data on the patient characteristics and the plan of management were collected at the beginning of the treatment using a pre tested structured questionnaire. During the follow up and at the end of the treatment, results of sputum microscopy and the treatment outcome were recorded. The patients who interrupted treatment for 2 months or more (defaulters) during the course of treatment were again investigated in the field. In the statistical analysis, patient characteristics of the defaulters were compared with the characteristics of patients who were cured.

Results: There were 22 defaulters giving a default rate of 4.59%. Being a male, poor educational background, having a casual job, regular smoking, and regular alcohol use were significantly associated with defaulting treatment ($p < 0.05$). Sinhalese had lower default rate than other ethnic groups. At the field investigation, 43% of the defaulters were found not living in the addresses given to the treatment providers. Field investigators have further reported that financial reasons, substance abuse, feeling well, and lack of family support also as causes of defaulting treatment.

Conclusion: Identification of risk factors by careful patient interview, early home visit, and monitoring of patient behaviour early in the course of treatment will help to predict whether adherence is likely to be a problem. So that, the treatment provider will be able to arrange a flexible and patient centered approach to ensure maximum adherence.

Key words: Non-adherence, Anti-TB Treatment, Sri Lanka

INTRODUCTION

Patients' adherence to anti TB treatment may be measured using either process oriented or outcome oriented definitions.[1] Default rate is an outcome oriented definition that may be used as an indicator of poor patient adherence to anti TB treatment. One of the main obstacles in achieving the best tuberculosis (TB) control is that patients do not complete full course of anti TB treatment. Review of annual reports of the National Programme for TB Control in Sri Lanka since year 2000 to 2008 reveals that default rate accounted for a large proportion of unfavourable treatment outcomes in any category of tuberculosis patients registered

Correspondence:
Dr. K.A.S. Jayawardena
National Programme for Tuberculosis Control and Chest Diseases
555/5, Elvitigala Mawatha, Narahenpita
Colombo 5, Sri Lanka
Email: kasjaya60@yahoo.com

for anti TB treatment. Non-adherence to correct treatment increases the risk of spreading the TB infection in the community and development of drug resistance in the patient.[2]

Sri Lanka had achieved a significant improvement in the tuberculosis control including a reduction of the default rate since introduction of the DOTS strategy to the national TB control programme in 1997. The default rate of 14.8 among new smear positive pulmonary TB patients registered in the year 2000 was gradually reduced up to 6.2 among those registered in 2005.[3,4] In the same category of patients the cure rate increased from 74.9 to 82.9 during the same period. The Fixed Dose Combination (FDC) chemotherapy was then introduced in 2005 to simplify administration of drugs and to improve the patient adherence to correct treatment regimen. FDC drugs too are said to be a quality combination of anti TB drugs. However, annual statistics of the national programme in subsequent years did not indicate a significant improvement of treatment success including reduction of default rate (Table 1). Reasons for non progression of treatment success may be multifactorial including patients continue to interrupt anti TB treatment.

Table 1. Selected outcome indicators of new sputum positive PTB cases registered in Sri Lanka 2000-2007

Year	Total number registered	Cure rate	Default rate
2000	4314	74.9	14.8
2001	4316	77.0	13.2
2002	4297	77.5	12.1
2003	3652	77.7	10.6
2004	4302	81.6	8.4
2005	4841	82.9	6.2
2006	4431	83.3	6.7
2007	4477	83.6	7.1

Source – National Programme for Tuberculosis Control and Chest Diseases

In practice it is the patient who is frequently blamed for interrupting the treatment. Literature review reveals that factors associated with adhering to anti TB treatment are multiple. These factors include patient characteristics, the relationship between healthcare provider and patient, social support, cultural influence, the treatment regimen and the health care setting.[1,5] It is interesting to investigate risk factors for non-adherences in the Sri Lankan context too. The aim of this study was to identify patient characteristics that associate with defaulting anti TB treatment.

METHODOLOGY

This was a prospective cohort study conducted in seven districts - Anuradhapura, Badulla, Colombo, Galle, Kegalla, Kurunegala, and Nuwara Eliya. The criterion used to select districts for the study was to have a proportionate representation of urban, rural, and estate populations in Sri Lanka in the study sample. All the selected districts had district TB clinics and qualified staff, laboratory facilities, and substantial number of patients attending for anti TB treatment. The study sample comprised of all new smear positive pulmonary tuberculosis patients registered for anti tuberculosis treatment (ATT) between 1.6.2008 and 31.8.2008. Ethical clearance was obtained from the Ethical Review Committee, Faculty of Medicine, University of Peradeniya, Sri Lanka. The patients were recruited for interview after obtaining informed consent to participate in the study.

An interviewer administered pre-tested structured questionnaire was used to collect data. Each patient's demographic profile, smoking, drug abuse and alcohol habits, details of past illnesses, and details of pre-treatment sputum examination results were recorded at the time of starting anti TB treatment. Patients who reported smoking more than 5 cigarettes a day for the last 5 years were considered as regular smokers and patients who reported consuming alcohol at least once a week for the last 6 months were considered as regular drinkers. Persons who have admitted themselves as current drug users (those who inhale or inject narcotic substances at present) were recorded as drug users. During the follow-up, sputum examinations *(DS X 2)* were done at two, five and six months of treatment. At the end of one year, treatment outcomes of all patients were recorded.

Patients who had been reported as having interrupted treatment continuously for more than 2 months during the course of treatment were identified as defaulters. These patients were

selected to analyse their personal characteristics that are associated with default. A field investigation was carried out using a check list to find out more information about these defaulters. The check list included a number of items to inquire whether the defaulter had given correct answers at the initial interview, level of the family support, was service provider unsupportive, financial status of the patient, and any other factors that contributed to the defaulting treatment.

Data collection was conducted by a group of trained data collectors under the direct supervision of the District Tuberculosis Control Officers (DTCO) of the respective district chest clinics. Each group comprised of a medical officer, a nursing officer and a field investigator (Public Health Inspector).

Patient characteristics that are associated with defaulting treatment were analyzed using the data collected by the questionnaire. In the statistical analysis, selected variables of defaulters were compared with the same variables of patients who had been reported as cured at the end of six months of treatment. The statistical tests (chi squared tests) were carried out using Minitab 14 statistical software. The $p < 0.05$ was considered as statistically significant. Findings of field investigations were summarized at the end of statistical data analysis.

RESULTS

A total of 462 new smear positive PTB patients had been enrolled from the seven districts during the study period. Four patients were excluded from the sample as they had been in different categories of treatment. Therefore the actual sample size was 458.

Personal characteristics of the patients

The patients of the sample were aged between 11 and 83 years with a mean of 43.6 years (SD 15.81Y). Majority of the patients were males (71.62%). Sinhalese constituted 75.8% of the entire sample. A majority of the respondents (58.73%) had studied up to the level of secondary school education while 7.4% had never gone to school. A majority of the patients (86.2%) reported living in the own residence and most of the others were living in a close relative's house. More than 80% of the patients were identified as economically active (employed, house wives or studying) at the time of diagnosis (Table 2). A majority (79%) of patients had expressed willingness to attend a local health centre for daily observed treatment while 6% had strongly refused to do so.

Table 2. Demographic profile of the patients (n = 458)

Variable	Number	Percentage
Age (Years)		
< 15	04	00.87
16 – 30	103	22.49
31 – 45	129	28.17
46 – 60	162	35.37
61 <	60	13.10
Sex		
Male	328	71.62
Female	130	28.38
Ethnicity		
Sinhalese	347	75.76
Tamil	52	11.35
Moor	52	11.35
Other	07	01.54
Highest level of education		
Not gone to school	34	07.42
Primary	143	31.22
Secondary	269	58.74
Higher	12	02.62
Current place of residence		
Permanent (own)	395	86.24
Street	04	00.87
Work place	12	02.62
Boarding house	07	01.53
Hostel	03	00.66
Relatives house	32	06.99
Prison	05	01.09
Occupation		
Permanent employee in government or private sector	68	14.85
Self employed	71	15.50
Casual labourer	91	19.87
Agricultural worker	40	08.73
Student	28	06.11
House wife	74	16.16
Retired or other	53	11.57
Un – employed	33	07.20

Nearly one third (31%) of the sample was identified as regular smokers and one fifth (20.5%) of the sample was identified as regular drinkers. A few patients (3.7%) had reported as having abused narcotic drugs (Table 3). Of the total, none of the female patients were identified as either regular smokers or alcohol and narcotic drug abusers.

Table 3. Distribution of patients by smoking, alcohol and drug abuse (n = 458)

Variable	Number	Percentage
Smoking		
Regular smoker	141	30.79
Never / occasional	317	69.21
Alcohol use		
Regular drinker	94	20.52
Never / occasional	364	79.48
Narcotic drug use		
Yes	17	3.71
No	426	93.01
Not known	15	03.28

A total of 77 (17.9%) patients had reported as having diabetes mellitus and 25 (5.81%) patients had reported as having chronic respiratory diseases (Table 4). Eighteen patients had reported as having multiple diseases which included diabetes (16), respiratory diseases (3), renal diseases (1), liver disease (1), and other diseases (14) in 2 or more combinations. Grading of the initial bacillary load in sputum samples was recorded in 426 patients and a majority (45.5%) had one plus (Table 5).

Table 4. Presence of co-morbidity reported by the patients (n = 430)

Disease	Number	Percentage*
Diabetes	77	17.91
Renal diseases	04	00.93
Liver diseases	01	00.23
Cancer	01	00.23
Respiratory diseases	25	05.81
Other	26	06.04
No disease	313	72.79

* Percentages will not add to 100 due to the presence of multiple responses

Table 5. Pre treatment sputum grading (n = 426)

Grading	Number	Percentage
Scanty	24	05.64
One plus	194	45.54
Two pluses	93	21.83
Three pluses	115	26.99

Four hundred and thirty six (95%) patients had been started on ATT under directly observed treatment (DOT). Of them, 84.63% had been put on daily DOT and the balance had been put on intermittent DOT on regular basis. Five percent had self-administered treatment. For a majority (67.2%) of the patients, DOT was arranged only for the intensive phase of the treatment. Government health care workers accounted for 86% of the DOT supervisors. The remaining 14% comprised of community volunteers and health workers in the private sector.

Three hundred and seventy eight (82.53%) patients were reported as cured and 28 patients (6.11%) were reported as treatment completed. Thus the overall success rate was 88.64%. A total of 51 (11.14%) patients were found to have had unfavourable treatment outcomes (death, defaulted and treatment failure) (Table 6).

Table 6. Treatment outcome of the sample (n=458)

Outcome	Number	Percentage
Cured	378	82.53
Treatment completed	28	06.11
Transferred out	01	00.22
Death	22	04.80
Defaulted	21	04.59
Failure	08	01.75
Total	458	100.00

Twenty one patients had defaulted treatment, giving a default rate of 4.59%. Among them, 10 (48%) had defaulted during the intensive phase of treatment. The Colombo district reported the highest (7.6%) default rate. According to the questionnaire all defaulters had been arranged DOTS in government health centres either daily (80%) or intermittent (20%) basis. Ninety percent of them had voluntarily consented that they could attend the treatment centre daily for treatment.

Of the total of 378 patients who reported as cured we selected those who had converted sputum at the end of the intensive phase of treatment and remained negative at the end of 5 and 6 months. There were 335 such patients. Personal characteristics of those patients were then compared with the defaulters using chi-squared test. Being a male, poor educational background,

having a casual job, and regular smoking and regular alcohol use were significantly associated with defaulting treatment (Table 7). Ethnic groups other than Sinhalese had relatively higher default rate while age, type of residence, initial sputum grading, and co-morbidity did not show a significant association with defaulting treatment.

The field investigation of defaulters revealed that 9 (43%) of the defaulted patients were not living in the addresses given to the district chest clinic and therefore were untraceable. Among the remaining defaulters, the reasons for default according to the opinions of the field investigators were financial constrains, substance abuse, feeling well, and lack of family support.

DISCUSSION

Sri Lanka has achieved global targets of case detection and treatment success in 2005. The case detection rate for 2007 was 85.6% and treatment success for the 2006 cohort of new smear-positive cases was 87%.[6] However, among the unfavourable treatment outcomes, proportion of default is substantial.[6] Adhering to daily treatment is not an easy task for a patient or a treatment provider. In this study, 21 patients had defaulted treatment for two months or more giving a default rate of 4.59. Being a male, being a casual employee, poor educational background, being a regular smoker and regular use of alcohol were significantly associated with defaulting treatment. None of the female patients of the sample had reported regular smoking and alcohol use, and only less than 1% had reported having casual jobs. These could be the main contributing factors for reporting significantly low default rate among female patients. A relatively higher percentage of patients in minority ethnic groups (Tamil and Moor) had defaulted treatment (Table 7). The place of residence, whether permanent or not, did not show a significant difference in relation to defaulting treatment. However, field investigation revealed that 43% of defaulted patients were not living in the addresses given to the district chest clinics. But, they all had indicated at the initial interview that they lived in permanent residences.

In a study conducted in Singapore, self-reported reasons for default had been described as financial difficulties, pressure of work, side effects, hospital admission, and physical hardships.[7] Poor interaction between DOT providers and patients had been demonstrated as a cause of poor treatment adherence in Nepal.[8] The present study showed that all defaulters had been arranged DOT in government health centres and nearly 50% of them had defaulted treatment during intensive phase of treatment. Health workers in the state health institutions are the main category of DOT providers in Sri Lanka. We observe that excessive use of state health centre based DOT carries certain disadvantages for patients. For example, some patients have to travel long distances for treatment on daily basis spending more money and experiencing physical hardships. Due to non-flexibility in time in the state health service, patients tend to lose their working time and therefore lose income when they attend for daily DOT. Similar problems of attending DOT centres were also reported in Nepal.[9] The same study further reported that social stigma attached to the disease, poor patient counseling by treatment providers, and consultation of traditional healers by TB patients also contributed to defaulting treatment. A study conducted in the Kandy district, Sri Lanka investigated the usefulness of recruiting trained family members as DOT providers in selected patients.[10] The results were encouraging in relation to treatment adherence. According to that study, the important prerequisites to ensure successful family DOT include correct selection of patients and DOT providers, giving adequate instruction for treatment adherence, and regular monitoring of drug intake by home visits.

We observed some limitations of our study. The present study did not systematically investigate the service factors associated with defaulting treatment. However some weaknesses of the service were identified while investigating defaulted patients in the field. For example, a large number of defaulters had not given proper information about their place of residence. This had not been identified by the treatment providers until those patients were investigated in the field for defaulting treatment. The calculated default rate for the study sample was 4.59. According to the

Table 7. Comparison of selected variables of defaulters with those of patients who cured within six months of treatment

Variables	Cured patients		Defaulters		χ^2	P value
	Number	%	Number	%		
Sex						
Male	233	92.46	19	7.54	4.18	**0.04**
Female	102	98.07	02	1.93		
Age						
< 45	171	95.54	8	4.46	1.32	0.25
≥ 45	164	92.65	13	7.35		
Ethnicity						
Sinhalese	262	95.27	13	4.73	2.98	0.08
Other	73	90.12	8	9.88		
Education						
≤ Primary	109	87.20	16	12.80	16.528	**0.000**
≥ Secondary	226	97.83	05	2.17		
Residence						
Permanent	289	94.13	18	5.87	0.005	0.943
Other	46	93.87	03	6.13		
Occupation						
Permanent	55	96.49	02	3.51	4.976	**0.026**
Casual	60	84.51	11	15.49		
Smoking						
Regular smoker	87	89.69	10	10.31	4.67	**0.031**
Other	248	95.75	11	4.25		
Use of alcohol						
Regular drinker	61	85.91	10	14.09	10.705	**0.001**
Other	247	96.14	11	3.86		
Initial sputum grading						
Scanty & one plus	173	94.53	10	5.47	0.226	0.635
Two & three pluses	139	93.29	10	6.71		
Co– morbidity						
Reported “yes”	87	96.66	3	3.34	1.56	0.212
Reported “no”	226	93.00	17	7.00		

annual statistics of the seven districts default rate of same category of patients in the same year was 7.1. One possibility for reporting higher default rate in the annual statistics may be due to miscounting of TB deaths as defaulters without investigating the reason for default. On the other hand health workers of the respective districts may have taken extra precautions to reduce the incidence of premature interruption of treatment by the study sample.

CONCLUSION

Demographic factors identified as risk factors for defaulting treatment are not amenable to intervention for immediate behaviour change. Identification of risk factors by careful patient interview, early field investigation (home visit), and monitoring of patient behaviour early in the course of treatment will help to predict whether adherence is likely to be a problem. So that, the treatment provider can plans a flexible patient centered approach to ensure maximum adherence. Service factors that associate with premature interruption of treatment by patients should also be investigated and corrected.

ACKNOWLEDGEMENTS

This study was funded by the GFATM. We are grateful to the patients who participated in this study. We sincerely thank the staff of the chest

clinics for collecting data and Dr. Hemantha Herath (consultant community physician, Ministry of Health) for technical advice.

REFERENCES

1. World Health Organization. Adherence to long term therapies. Evidence for action. Geneva: World Health Organization 2003:123-30.
2. World Health Organization. Treatment of Tuberculosis. Guidelines for national programmes. Geneva: World Health Organization 2003:47-52.
3. National Programme for Tuberculosis Control and Chest Disease. Administration Report 2001. Colombo: Ministry of Health, Sri Lanka.
4. National Programme for Tuberculosis Control and Chest Disease. Annual Report 2006. Colombo: Ministry of Health Sri Lanka.
5. Sumartojo E. When tuberculosis treatment fails. A social behavioral account of patient adherence. American Review of Respiratory Disease 1993;147:1311- 20
6. National Programme for Tuberculosis Control and Chest Disease. Annual Report 2007. Colombo: Ministry of Health Sri Lanka.
7. Chee CBE, Boudville IC, Chan SP, Zee YK, Wang YT. Patient and disease characteristics, and outcome of treatment defaulters from Singapore TB control unit – a one year retrospective survey. Int J Tuberc Lung Dis 2000;4:496–503.
8. Mirsha P, Hasan EH, Sabroe S, Kafle KK. Adherence is associated with the quality of professional – patient interaction in Directly Observed Treatment Short-course, DOTS. Patient Education and Counseling 2006;63:29-37.
9 Lamsal DK, Lewis OD, Smith S, Jha N. Factors related to defaulter and treatment failure of tuberculosis patients in the DOTS program in the Sunsary district of Eastern Nepal. SAARC J Tuber Lung Dis HIV/AIDS 2009; VI:25-30.
10 Jayawardena KAS, Medagedara D. Involving family members in the delivery of TB care in the Kandy district, Sri Lanka. SAARC J Tuber Lung Dis HIV/AIDS 2009; VI:12–15.

SIMPLE AND RAPID LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF RIFABUTIN IN PLASMA

Hemanth Kumar AK, Sudha V, Ramachandran G

National Institute for Research in Tuberculosis, Chetput, Chennai, India

ABSTRACT

A high performance liquid chromatographic method for determination of rifabutin in human plasma was developed. The method involved deproteinisation of the sample with acetonitrile and analysis of the supernatant using a reversed-phase C_{18} column (250mm) and UV detection at a wavelength of 265nm. The assay was specific for rifabutin and linear from 0.025 to 10.0µg/ml. The relative standard deviation of intra- and inter-day assays was lower than 10%. The method was able to remove interfering materials in plasma, yielding an average recovery of rifabutin from plasma of 101%. Due to its simplicity, the assay can be used for pharmacokinetic studies of rifabutin.

INTRODUCTION

Patients' adherence to anti TB treatment may be he rifamycin class of compounds, rifampicin, rifapentine and rifabutin play a key role in the treatment of tuberculosis. The rifamycins are known to be potent inducers of the hepatic CYP450 enzyme system, but vary in their potential as CYP450 inducers, with rifampicin being most potent, rifapentine intermediate, and rifabutin being much less active. Rifabutin has been shown to be as effective against tuberculosis as rifampicin[1,2] and is the preferred rifamycin in situations were rifampicin is contraindicated, such as during concomitant use of protease inhibitors.[3]

A few methods have been described for estimation of plasma rifabutin. The method of Lewis et al involves a cumbersome sample preparation procedure, using one ml plasma[4], while other methods require special columns[5,6] and column switching.[7] We developed and validated a simple and rapid assay procedure for estimation of rifabutin in plasma using rifampicin as an internal standard.

Correspondence:
Dr. Geetha Ramachandran
Scientist 'C
Department of Clinical Research
National Institute for Research in Tuberculosis
(formerly Tuberculosis Research Centre)
Mayor V.R. Ramanathan Road
Chetput, Chennai-600 031, India
E-mail: geetha202@rediffmail.com

METHODOLOGY

Pure rifabutin and rifampicin powders from Sigma Chemical Company, MO, USA, Acetonitrile (HPLC grade) from Merck (India) and potassium dihydrogen orthophosphate from Qualigens (India) were used. Deionized water was processed through a Milli-Q water purification system (Millipore, USA). Pooled human plasma was obtained from Lions Blood Bank, Chennai, India.

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of two pumps (LC-10ATvp), diode array detector (SPD-M10Avp) and auto-sampler with built-in system controller (SIL-HTA). Class VP-LC workstation was used for data collection and acquisition. The analytical column was a C_{18}, 250 x 4.6mm ID, 5µ particle size (Lichrospher 100 RP-18e, Merck, Germany) protected by a compatible guard column.

The mobile phase consisted of 50mM phosphate buffer, pH 4.2 (adjusted with 1N HCl) and acetonitrile (53:47 v/v). Prior to preparation of the mobile phase, the phosphate buffer and acetonitrile were degassed separately using a Millipore vacuum pump. The UV detector was set

at a wavelength of 265nm. The chromatogram was run for 10 minutes at a flow rate of 1.2 ml/min at ambient temperature. Unknown concentrations were derived from linear regression analysis of the peak height ratios (analyte/internal standard) vs. concentration curve. The linearity was verified using estimates of correlation coefficient (r).

A stock standard (1 mg/ml) was prepared by dissolving rifabutin in methanol. The working standards of rifabutin in concentrations ranging from 0.025 to 10.0µg/ml were prepared in human plasma.

To 200 µl each of calibration standards and test samples, 20 µl of rifampicin (internal standard) was added at a concentration of 20µg/ml. This was mixed with 300 µl of acetonitrile, the contents were vortexed vigorously, and centrifuged at 10,000 rpm for 10 minutes. Three hundred microlitres of the clear supernatant was taken into the test tube, evaporated to dryness in a nitrogen evaporator (turbo vapor) for 20 minutes. The dried residue was reconstituted in 200µl of mobile phase; 100µl was injected into the HPLC column

The accuracy and linearity of rifabutin standards were evaluated by analysing a set of standards ranging from 0.025 to 10.0µg/ml. The within day and between day variations were determined by processing each standard concentration in duplicate for six consecutive days.

In order to evaluate the precision of the method, three different concentrations of rifabutin (1.3, 5.5 and 10.0µg/ml) were prepared in pooled human lasma and analysed in duplicate on three consecutive days.

Varying concentrations of rifabutin (0.3, 4.5 and 9.0µg/ml) were prepared in drug-free human plasma and extracted as described above along with the internal standard. The percentage of the drug recovered from the plasma samples was determined by comparing the peak height ratio after extraction with those of unextracted methanolic solutions containing same concentrations of rifabutin as in plasma. Recovery experiments were carried out on three different occasions.

Interference from endogenous compounds was investigated by analysing blank plasma samples obtained from six each of male and female subjects. Interference from certain anti-tuberculosis drugs such as, isoniazid, pyrazinamide, rifapentine, ethambutol, streptomycin and certain antiretroviral drugs, namely, nevirapine, efavirenz, zidovudine, tenofovir, didanosine, stavudine, lamivudine, lopinavir, ritonavir, indinavir and saquinavir at a concentration of 10µg/ml was also evaluated.

Serial blood samples at 2, 4 and 6 hours post dosing were drawn in heparinized containers from a HIV-infected child with tuberculosis (age – 12 years; sex – male; body weight – 22kg) who was receiving rifabutin (150mg thrice weekly) as part of the treatment regimen. All the blood samples were centrifuged and plasma was separated and stored at -20°C until assay. Estimation of plasma rifabutin was undertaken within 24 to 48 hours of blood collection. Informed written consent was obtained from the parent before blood draws were made.

RESULTS

Under the chromatographic conditions described above, rifabutin was well separated as seen in the representative chromatograms (Figure 1a,b). The retention times of the internal standard and rifabutin were 2.5 and 8.6 minutes respectively. Blank plasma samples did not give any peak at the retention times of rifabutin (Figure 1c). Chromatogram of a patient's sample containing rifabutin and extracted following the same procedure as standards is shown in Figure 1d. The lowest concentration of rifabutin gave a discrete peak at 8.7 minutes (Figure 1a).

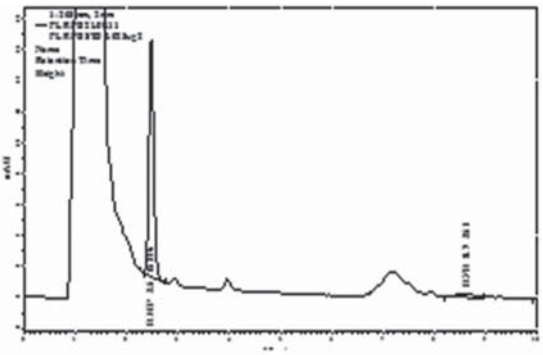


Figure 1a: Chromatogram of extracted rifabutin plasma standard 0.025 µg/ml (contains internal standard - 20µ of rifampicin added at a concentration of 20µg/ml)

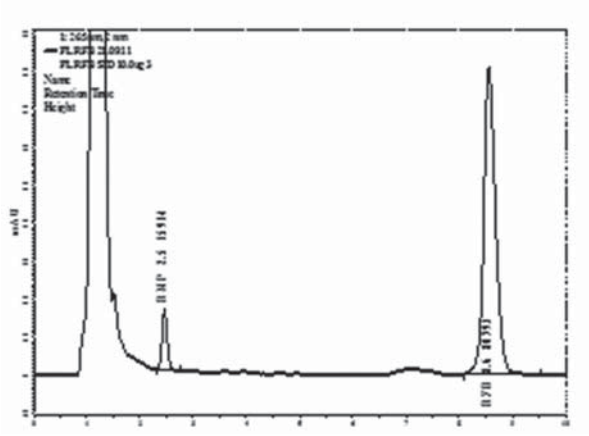


Figure 1b: Chromatogram of extracted rifabutin plasma standard 10.0 µg/ml (contains internal standard - 20µ of rifampicin added at a concentration of 20µg/ml)

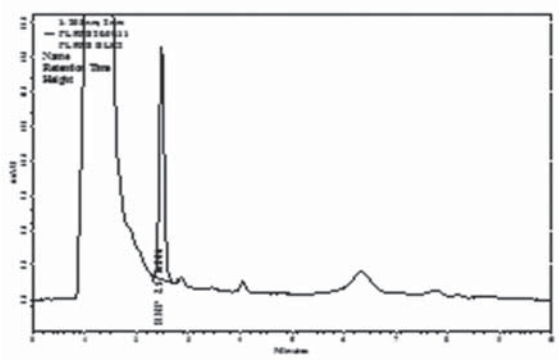


Figure 1c: Chromatogram of extracted blank plasma (contains internal standard - 20µ of rifampicin added at a concentration of 20µg/ml)

No endogenous substances or first-line anti-tuberculosis drugs such as isoniazid, pyrazinamide, ethambutol, streptomycin or antiretroviral drugs such as nevirapine, efavirenz, zidovudine, tenofovir, didanosine, stavudine, lamivudine, lopinavir, ritonavir, indinavir and saquinavir interfered with the rifabutin chromatogram.

Rifabutin concentrations ranging from 0.025-10.0 µg/ml were checked for linearity. The calibration curve parameters of rifabutin from six individual experiments for standard concentrations ranging from 0.025 to 10.0µg/ml showed a linear relationship between peak height ratio and concentrations. The mean (± SD) correlation coefficient, slope and intercept values were 0.9993 ± 0.0008, 0.6685 ± 0.2758 and -0.0225 ± 0.016 respectively. The linearity and reproducibility of the various standards used for constructing calibration graphs for plasma rifabutin are given in Table 1. The within-day and between-day percent variation (CV) for standards

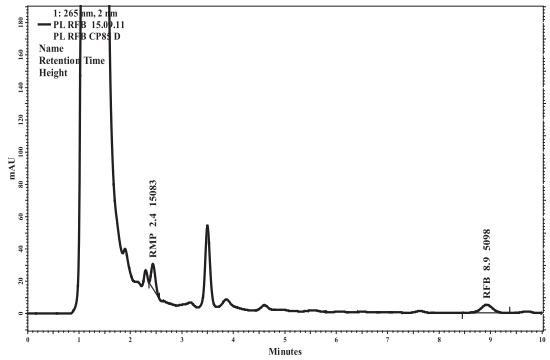


Figure 1d: Chromatogram of extracted rifabutin from a patient's plasma sample (contains internal standard - 20 µl of rifampicin added at a concentration of 20µg/ml)

containing 0.025 to 10.0µg/ml ranged from 1.0 to 9.1% and 2.1 to 9.1% respectively.

The reproducibility of the method was further evaluated by analysing three plasma samples containing different concentrations of rifabutin. The RSD for these samples ranged from 2.7 to 5.3% (Table 2). The % variations from the actual concentrations ranged from 98 to 108%. The Limit of Quantification (LOQ) was 0.025µg/ml. The percent recovery of rifabutin from plasma ranged from 98 to 100%. The concentrations of rifabutin in serial blood samples collected at 2, 4 and 6 hours post dosing were 0.86, 0.82 and 0.17 µg/ml; the peak concentration being 0.86µg/ml.

Table 1. Linearity and reproducibility of plasma rifabutin standards

Standard Concentration (µg/ml)	Mean peak height ratio ± SD (% CV)	
	Within day (n=6)	Between day (n=6)
0.025	0.011 ± 0.001 (9.1)	0.01 ± 0.001(8.9)
0.05	0.025 ± 0.002 (7.1)	0.021 ± 0.002 (9.1)
0.1	0.047 ± 0.002 (4.5)	0.043 ± 0.003 (7.8)
0.5	0.240 ± 0.002 (1.0)	0.231 ± 0.010 (4.5)
1.0	0.488 ± 0.019 (3.9)	0.470 ± 0.022(4.8)
2.5	1.174 ± 0.052 (4.4)	1.163 ± 0.049(4.3)
5.0	2.438 ± 0.090 (3.7)	2.377 ± 0.147(6.2)
10.0	4.926 ± 0.163 (3.3)	4.926 ± 0.103(2.1)

Table 2. Precision of plasma rifabutin assay

Actual Conc. (µg/ml)	Found Conc. (µg/ml) Mean ± SD (% RSD)
1.3	1.27 ± 0.06 (4.6)
5.5	5.38 ± 0.29 (5.3)
10	9.98 ± 0.27 (2.7)

DISCUSSION

A few HPLC methods have been described to measure rifabutin concentrations in plasma for pharmacokinetic studies. The method described by Lewis et al requires one ml plasma, and a lengthy sample pretreatment procedure[4], while the methods of Lau et al and Gatti et al have employed special columns, which could be relatively expensive.[5, 6] The method of Bartels et al requires column switching, which could be cumbersome.[7] The present method has the advantages of being rapid (run time is only 10 minutes), employing a simple one-step sample preparation step and using a small sample volume (300 microlitres), without any loss of analyte. The use of rifampicin as internal standard helped in monitoring the recovery of rifabutin from plasma.

The method was quite robust as evidenced by a discrete peak denoting rifabutin. The percent variations (within-day and between-day) for the standards were below 10%. The method reliably eliminated interfering material from plasma, yielding an acceptable range of recovery. Further, data from the accuracy and precision experiments yielded satisfactory results.

In view of its potent antimycobacterial activity, rifabutin is likely to be used in the treatment of tuberculosis along with anti-tuberculosis and antiretroviral drugs. It therefore becomes necessary to rule out interference of both these classes of drugs in the assay of rifabutin and establish the specificity of the method. The method was highly specific for rifabutin; drugs such as isoniazid, pyrazinamide, ethambutol, streptomycin or antiretroviral drugs such as nevirapine, efavirenz, zidovudine, tenofovir, didanosine, stavudine, lamivudine, lopinavir, ritonavir, indinavir and saquinavir or any endogenous substance interfered in the assay.

When this method was applied to estimate rifabutin in plasma samples collected from a child, we obtained a peak concentration of 0.86µg/ml, which was within the therapeutic range of rifabutin.[8] Thus the method spans the range of clinical interest and could be applied to pharmacokinetic studies in both adults and children.

In conclusion, a sensitive, specific and validated method for quantitative determination of rifabutin in plasma is described. This simple, rapid, accurate and reproducible method utilises a single step extraction. The chromatogram yields a well-resolved peak for rifabutin with good intra- and inter-day precision. The easy sample preparation and small sample size makes this assay highly suitable for pharmacokinetic studies of rifabutin in tuberculosis patients.

REFERENCES

1. Schwander S, Rusch-Gerdes S, Mateega A, Lutalo T, Tugume S, Kityo C, et al. A pilot study of antituberculosis combinations comparing rifabutin with rifampicin in the treatment of HIV-1 associated tuberculosis. A single-blind randomized evaluation in Ugandan patients with HIV-1 infection and pulmonary tuberculosis. Tubercle Lung Dis. 1995;76:210-18.

2. Gonzalez-Montaner LJ, Natal S, Yonchaiyud P, Olliaro P. Rifabutin for the treatment of newly-diagnosed pulmonary tuberculosis: a multinational, randomized, comparative study versus Rifampicin. Rifabutin Study Group. Tubercle Lung Dis. 1994;75:341-47.

3. Burman WJ, Gallicano K, Peloquin CA. Therapeutic implications of drug interactions in the treatment of HIV-related tuberculosis. Clin Infect Dis. 1999;28:419-30.

4. Lewis RC, Hatfield NZ, Narang PK. A sensitive method for quantitation of rifabutin and its desacetyl metabolite in human biological fluids by HPLC. Pharm Res. 1991;8:1434-40.

5. Lau YY, Hanson GD, Carel BJ. Determination of rifabutin in human plasma by high performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Appl. 1996;676:125-30.

6. Gatti G, De Pascalis CR, Miletich F, Casazza R, Bassetti D. Specific high performance liquid chromatography assay for determination of rifabutin plasma concentration following Extrelut column extraction. J Chromatogr B Biomed Sci Appl. 1999;728:233-39.

7. Bartels H, Bartels R. Determination of rifabutin by high performance liquid chromatography using on-line concentration and column switching. J Chromatogr B Biomed Appl. 1996;686:235-40.

8. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 2002;62:2169-83.

Case study

EXCESS OF PLASMA CELLS WITH PSEUDO-GAUCHER CELLS IN A CASE OF TUBERCULOSIS – A CASE REPORT

Palta A, Dhiman P, Ram J

Department of Pathology & Medicine, Govt. Medical College & Hospital , Chandigarh, India.

ABSTRACT

This report describes a case of 50 year old woman fever and bony pains with lytic lesion in skull. A polyclonal band was seen in γ region on serum electrophoresis. Bone marrow examination showed excess of plasma cells along with many Pseudo- Gaucher cells. The diagnosis of chronic inflammatory reaction was made. Although stain for AFB was negative, the patient responded to antitubercular treatment. The presence of pseudo-gaucher cells along with plasmacytosis is a rare finding in tuberculosis.

Key words: Tuberculosis, Pseudogaucher Cell

INTRODUCTION

Tuberculosis, one of the oldest diseases known to affect humans, is a major cause of death worldwide.[1] According to World Health Organization (WHO), there were an estimated 8.8 million incident cases of TB globally in 2010, 1.1 million deaths among HIV-negative cases of TB and an additional 0.35 million deaths among people who were HIV-positive. India alone accounted for an estimated one quarter (26%) of all TB cases worldwide with 2.0–2.5 million new cases of TB in 2010.[2]

A variety of haematological alterations ranging from various cytopenias to leukaemoid reaction and even frank leukemia in association with tuberculosis have been reported.[3] It can present with anemia, neutrophilic leucocytosis, monocytosis or lymphocytosis. Some patients may show hypoplasia of one or more cell lines, associated with anemia, neutropenia and thrombocytopenia.[4] Here we describe a case of tuberculosis in which bone marrow revealed cluster of pseudo-Gaucher cells with plasma cells making it difficult to exclude the diagnosis of multiple myeloma.

Correspondence:
Dr Pratibha Dhiman
Department of Pathology
Government Medical College-32A
Chandigarh, India.
E-mail: dhimandrpratibha@yahoo.com

CASE REPORT

A 50-year old female presented with complaints of fever, cough and bony pains since 5 weeks to our hospital. On physical examination no lymphadenopathy and hepatosplenomegaly noted. The hemogram showed anemia with a hemoglobin of 5.3 g/dl, high TLC of 14,000/µl with DLC of P_{83} L_{13} M_{02} E_{02} and platelet count of 2.4 X $10^{5/}$ µl. ESR was 60mm/hr. Both blood & urine cultures were negative. Skeletal survey showed lytic lesion in skull. CT scan revealed focal cystic lesion with fibrotic change in upper zone of right lung along with effusion. CT abdomen did not show any abnormality. Serum electrophoresis for M band showed a polyclonal band in γ region (Figure 1). Urine for Bence jones proteins (BJP) was negative. Serology for HIV, HBsAg and antiHCV were negative. Bone marrow aspiration and trephine biopsy were done for persistent anemia and pyrexia of unknown origin.

The bone marrow examination showed hypercellular marrow with 23% plasma cells along with many binucleate forms (Figure 2). Many scattered large cells with small and dense nuclei with abundant amount of foamy granular cytoplasm were identified. Biopsy also showed collection of these histiocytes with reticular cytoplasm (Figure 3). These cells were negative for Perl's stain conforming to the morphology of Pseudo-gaucher

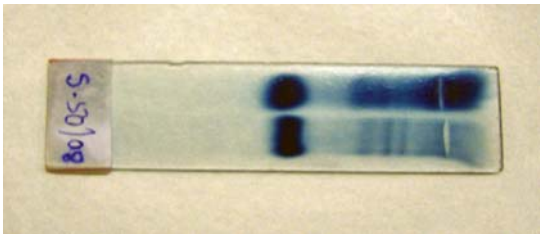


Figure 1: Serum electrophoresis showing polyclonal band in gamma globulin region

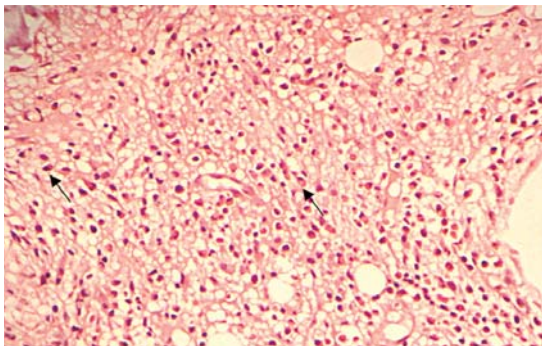


Figure 2: Trephine biopsy showing excess of plasma cells (Arrow head) (H&Estain,400X)

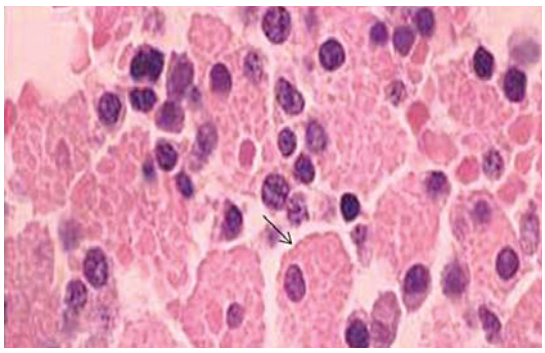


Figure 3: Trephine biopsy showing Pseudo-Gaucher cells (Arrow head) (H&Estain,1000X)

cells. There was focal area of fibrosis (reticulin stain) (Figure 4). Although stain for AFB was negative and the count of plasma cells was in range of criteria for multiple myeloma, in view of polyclonal band in γ region and urine negative for BJP ,a possibility of chronic inflammatory reaction was suggested. As per data, AFB microscopy has 40-60% sensitivity.[1] The examination of pleural fluid show high protein level along with high ADA and increased lymphocytes. Patient was given ATT. Patient became afebrile and follow up hemogram after one month of treatment revealed hemoglobin of 8.2g/dl with single whole blood transfusion which she has received before starting ATT.

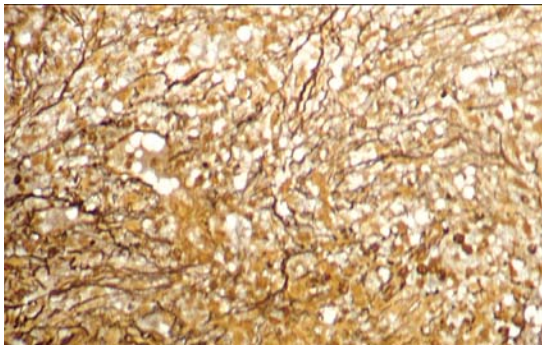


Figure 4: Trephine biopsy showing focal fibrosis (Reticulin stain, 400X)

DISCUSSION

We, here present a case of tuberculosis in immunocompetent patient associated with pseudogaucher cells and an excess of plasma cells. Typical Gaucher cells have a characterstic wrinkled tissue paper appearance of the cytoplasm as a result of deposition of glucocerebroside. These cells can be upto 60µm in diameter with fibrillary structures in the cytoplasm.[5] In the presence of cytopenias, hepatosplenomegaly, and typical Gaucher cells in the bone marrow, the diagnosis of Gaucher disease is made. In our case patient was normal before the presentation of tuberculosis. Bone marrow cells with morphological characterstics similar to Gaucher cells have been rarely described in chronic myeloid leukemia, Hodgkin's lymphoma, thalassemia and multiple myeloma.[6]

Solis et al reported the first case of pseudo-gaucher cells in Mycobacterium avium – intercellulare infection in HIV infected patient. They noted massive infiltration of lamina propria of the bronchial mucosa & bone marrow with foamy histiocytes.[7] Later link et al noted another case of tuberculosis with pseudo–gaucher cells in bone marrow, lymph nodes, spleen & lungs.This patient had pulmonary tuberculosis and the HIV status was unknown. In this case stain for AFB and Periodic acid Schiff staining were negative.[8]

In our case also these cells were negative for Periodic acid Schiff and Perl's stain. Based on immunologic studies, the cell of origin of the pseudo-Gaucher cell appears to be the monocyte.

On electron microscopy, Gaucher cells contain tubular cytoplasmic inclusions, which are absent in pseudo-Gaucher cells.[6]

Reactive plasmacytosis have been described in patients with liver disease, chronic infections, connective tissue diseases and carcinoma .[9] An excess of plasma cells have been described in tuberculosis but upto the extent of 23% have not been described in the literature till date to the best of our knowledge.

CONCLUSION

An excess of plasma cells along with pseudo-Gaucher cells in bone marrow, should be dealt with caution. Pseudo-Gaucher cells might be the morphological hallmark of mycobacterial infection in immunodeficiency but otherwise also AFB stain should be performed. Before thinking on the lines of Multiple myeloma possibility of tuberculosis should be considered and patient should be worked up accordingly.

REFERENCES

1. Raviglione MC, Brien RJ. Tuberculosis. Fauci AS, Braunwald E, Kasper DL, editors. Harrison's Principles of Internal Medicine. 17th ed. New York: McGraw Hill. 2008;1006-7.
2. Global Tuberculosis Control: The burden of disease caused by TB; 2011. WHO Technical Report.
3. Chandra, J., Marwaha, R.K., et al. Pancytopenia and Leukemoid Reactions in Tuberculosis. Indian J. Paed; 1986;53:665.
4. Fleming AF, De Silva PS. Hematological Diseases in Tropics. Cook GC, Manson M, Zumla A, editors. Manson's Tropical Diseases. 22nd ed. Edinburgh: Saunders. 2008;.211-12.
5. Dunn P, Kuo M-C, Sun C-F. Pseudo-Gaucher cells in mycobacterial infection: A Report of Two Cases. J Clin Pathol. 2005;58:1113-4.
6. Shenjere P, Roy A, Banerjee S. Pseudo-Gaucher Cells in Multiple Myeloma. Int. J Surg. Pathol. 2008;16:176-9.
7. Solis OG, Belmonte AH, Ramaswamy G, et al. Pseudogaucher Cells in Mycobacterium Avium Intracellulare Infection in Acquired Immune Deficiency Syndrome (AIDS). Am J Clin Pathol. 1986;85:233-5.
8. Links TP, Karrenbeld A, Steensma JT, et al. Fatal respiratory failure caused by pulmonary infiltration by Pseudo-Gaucher cells. Chest 1992;101:265-6.
9. Dispenzieri A, Gertz MA, Therneau TM, et al. Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clin Proc 2001;76:476–487.

LIMITED WEGENER'S GRANULOMATOSIS AS A SOLITARY PULMONARY NODULE - A CASE REPORT

Mishra M[1], Singla R[2], Dewan RK[3]

[1] Subharti Medical College, Meerut – 250 005, U.P., India
[2,3] LRS Institute of TB & Respiratory Diseases, Sri Aurobindo Marg, New Delhi -110 030, India

ABSTRACT

Limited Wegener`s Granulomatosis (WG) has been infrequently reported from India so far. We report a case of Limited WG in a 60 year old male, who presented to us with productive cough and hemoptysis, and a right upper lobe mass lesion on chest roentgenogram. He was previously treated outside as Pulmonary Koch's, without any improvement. We diagnosed the patient on histo-pathological examination of right upper lobectomy specimen. He was administered five pulses of intravenous cyclophosphamide therapy plus oral corticosteroids, with significant symptomatic improvement. He was given oral methotrexate and tapering doses of corticosteroids in follow up.

Key words: Limited Wegener's Granulomatosis, Solitary Pulmonary Nodule

INTRODUCTION

Wegener's granulomatosis is a systemic necrotizing granulomatous vasculitis of unknown etiology, which usually consists of a triad of necrotizing angiitis of the upper and lower respiratory tract and focal glomerulonephritis of the kidney (classic/severe form)[1,2], but may virtually involve any organ system. The concept of limited form of Wegener's was introduced in 1966 by Carrington and Liebow[3] to identify otherwise classic vasculitis lacking renal involvement. Limited Wegener's is said to carry a better prognosis than classic disease[2,4], but it may be extremely challenging to recognize and diagnose. Anti-neutrophil cytoplasmic antibodies (ANCAs) have a high degree of association with WG. Their positive predictive value ranges between 19-66%[2,5] c-ANCA is more specific than p-ANCA and is positive in more than 90% of patients with active WG.[6] In Limited Wegener's, the sensitivity of c-ANCA is 65-70%.[6] Those patients of WG who are p-ANCA positive (<5%)[7] have fewer organ involvement than those with c-ANCA.

Correspondence:
Dr. Rupak Singla
Head, Department of TB & Respiratory Diseases LRS Institute of TB & Respiratory Diseases
Sri Aurobindo Marg, New Delhi-110030, India.
Phone: (+91)9891168908

CASE REPORT

We report the case of a 60 year old normotensive, non-diabetic male, ex-smoker, who was referred to us as a suspected case of right upper lobe lung abscess/aspergilloma for surgical opinion. This gentleman presented to us with complaints of shortness of breath on exertion for last three years, cough with expectoration of purulent sputum for last ten months, and streaking of sputum, off and on, for last ten months.

Previously, about 30 years back, the patient took Anti-Tubercular Therapy (ATT) for left pleural effusion for one year. For chronic cough, streaking of sputum and suggestive radiological picture, he again received ATT from outside for ten months, nearly a year before admission to our institute in November 2009.

CT Thorax (Figure 1) revealed a well defined cystic lesion 3.74cm x 4.69cm in the apical segment of right upper lobe with mild surrounding consolidation, with an eccentric air shadow seen within it. No mediastinal lymph nodes were seen. IgG-hydatid cyst (2.37 U/L), total IgE (189 IU/L) and serum

precipitins against Aspergillus were negative. A CT guided FNA of the mass lesion revealed necrotic cell debris and degenerated inflammatory cells; no epithelioid cells/AFB were visualized.

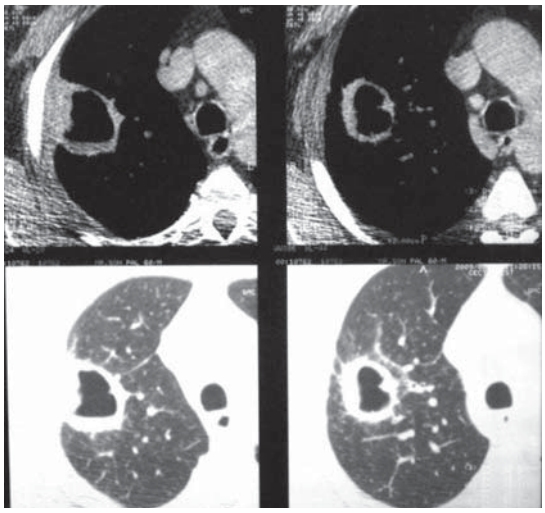


Figure 1: CT-Thorax showing well defined cystic lesion 3.74cm x 4.69cm in the apical segment of right upper lobe

With the above previous investigations that were performed outside, the patient was referred to our institute for further evaluation. The general physical examination was unremarkable, and systemic examination did not reveal any significant abnormality, except for occasional wheeze heard upon auscultation of the chest. There was no history of dysuria, oliguria, hematuria; allergy, nasal discharge, recurrent ENT infection, hearing difficulty, skin or eye lesions; joint pains, hematemesis, malena, abdominal pain; orthopnea, palpitations, or swelling of feet / face. All routine baseline investigations viz. hemogram, liver function tests, renal function tests, electrolytes, HIV/HBsAg/HCV and urine routine/microscopic examination were within normal limits. The chest x-ray (Figure 2) showed a mass lesion with an air-fluid level in the right upper lobe. A right upper lobectomy was performed which revealed a well-defined cystic lesion in right upper lobe. Histo-pathological examination of the resected specimen was suggestive of necrotizing granulomatous lesion associated with vasculitis, possibly Wegener`s Granulomatosis. c-ANCA was weakly positive. The patient was diagnosed as a case of limited Wegener`s Granulomatosis.

Between December 2009 and April 2010, the patient was administered five cycles of monthly pulse cyclophosphamide therapy with injection MESNA [2-Mercapto Ethane Sulfonate sodium (NA)] and oral corticosteroids. Thereafter he was given oral methotrexate with tapering dose of corticosteroids. Over the course of his treatment, he showed significant symptomatic relief. Subsequently he was lost to follow-up after six months.

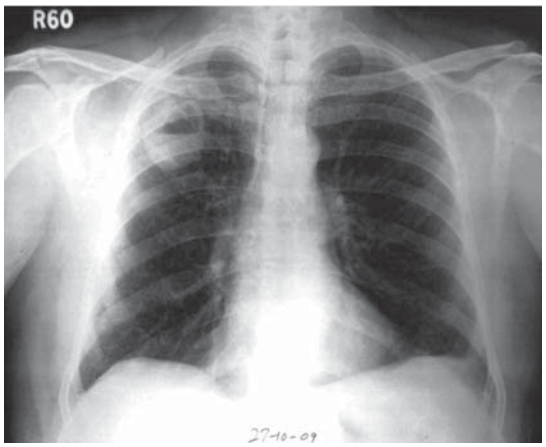


Figure 2: Chest X-ray PA view showing mass lesion with an air-fluid level in the right upper lobe

DISCUSSION

WG is the most frequent ANCA–associated vasculitis. Clinically, it is characterized by the triad of upper airway disease (otitis, ulcerations, sinusitis, bony deformities, and tracheal or bronchial stenosis), lower respiratory tract involvement (chest pain, cough, dyspnoea, and hemoptysis), and glomerulonephritis, which occurs in 50-90% patients.[8-11] Upper airway disease is the most common presenting feature of WG, sinusitis being the most frequent initial presentation in about half to two thirds of patients.[12,13]. Pulmonary involvement occurs in 45% of patients at presentation and in more than 90% of patients during the course of the disease. In 9% of the cases, lung is the only organ affected.[14] Lung involvement may range from subclinical changes (found as incidental findings in high-resolution CT scans), to the finding of diffuse alveolar hemorrhage in the most devastating cases.[15] In the mid-1960s, limited forms of WG were recognized. Limited disease, in contrast to the severe form, includes manifestations of WG that pose no immediate threat to either the patient's life or the function of a vital organ.[4] Advanced age, severe renal involvement, alveolar hemorrhage and c-ANCA positivity correlate with a worse outcome.[16-18]

Unlike other granulomatous disorders, eg. sarcoidosis and post-primary tuberculosis, WG has a tendency to involve the lower portions of the lungs.[3] Radiological presentation may be in the form of interstitial, mixed, or alveolar infiltrates, or nodular or cavitary disease.[10,11,14,15] The most common radiographic findings include pulmonary infiltrate (67%) and nodules (58%).[12] Nodules are predominantly subpleural and are cavitated in nearly 50% of cases.[19] In limited forms, the pulmonary lesions are usually multiple and bilateral, often simulate primary or metastatic neoplasms of the lung, but may suggest infectious granulomatous disease. Our case presented as a solitary pulmonary nodule.

Traditional initial therapy of WG consists of daily oral cyclophosphamide-corticosteroid combination therapy. This treatment has been quite effective in inducing remission in >90% of patients. However, in patients who have limited disease, monthly intravenous cyclophosphamide pulse therapy can be effective and minimize the side effects.[20-22] Pulse therapy is given in conjunction with prednisone, usually starting at 1 mg/kg/day for at least 1 month, then tapering by 5 to 10 mg/week until prednisone is stopped or the patient is placed on maintenance therapy of 5-15 mg every other day. Other therapies for WG include methotrexate and prednisone, which is an alternative for patients with active but not immediately life threatening disease and normal or near-normal renal function.[23,24] Prednisone alone is not a recommended therapy for WG.[22]

Our patient illustrates an unusual presentation of histo-pathologically proven limited WG, in which the pulmonary lesion was located in the apical segment of right upper lobe as a solitary pulmonary nodule. Surgery (right upper lobectomy) was performed to establish a tissue diagnosis. No evidence of systemic vasculitis could be found. The differential diagnoses that we considered in our patient and their possible respective reasons for exclusion were:

1. Pulmonary tuberculosis - no improvement with ATT.
2. Lung abscess - no history of foul-smelling copious expectoration, any dental infection/ procedure, and no improvement with systemic antibiotics.
3. Fungal infection (aspergilloma) - total IgE and serum precipitins against aspergillus species were negative.
4. Neoplasm of the lung – chronic illness, without a history of weight loss and no evidence of metastases.

Cases of WG presenting with a solitary pulmonary nodule have been described, but uncommonly.[25] This case report thus represents a rather uncommon presentation of limited Wegener's granulomatosis as a solitary pulmonary nodule in the apical segment of right upper lobe.

CONCLUSION

Limited WG is an uncommon entity that may pose significant diagnostic challenges as it closely mimics other more common disorders like pulmonary tuberculosis, lung cancer, lung abscess, etc. An improved knowledge of clinical and imaging features of WG could help the clinician reach an earlier diagnosis. Also, once correctly identified, the response to treatment as well as prognosis of limited WG is better as compared to severe WG. We therefore recommend having a high index of suspicion for this form of the disease, especially when the patient is inadequately responding to other forms of treatment. Future areas of research may include the investigation of other therapeutic regimens/alternative forms of therapy that could match the success of cyclophosphamide and at the same time is free from concerns about toxicity arising from prolonged usage. The difference between limited and severe forms of WG with regard to sex, age, likelihood of recurrent disease, risk of damage in certain organ systems, and, possibly, etiologic factors also needs further clinical investigations in order to explore the pathogenesis, therapeutic, and prognostic differences between these two subsets.

REFERENCES

1. Stone JH. Limited versus severe Wegener's granulomatosis: baseline data on patients in the Wegener's granulomatosis etanercept trial. Arthritis Rheum 2003;48:2299-309.
2. Mlika M, Ayadi-Kaddour A, Marghli A, Kilani T, El Mezni F. Pulmonary localization revealing Wegener's granulomatosis. Scientific World Journal 2010;10:806–10.

3. Carrington CB, Liebow A. Limited forms of angiitis and granulomatosis of Wegener's type. Am J Med 1966;41:497-527.

4. Reinhold-Keller E, Beuge N, Latza U, de Groot K, Rudert H, Nölle B et al. An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. Arthritis Rheum 2000;43:1021–32.

5. Shafiei K, Luther E, Archie M, Gulick J, Fowler MR. Wegener granulomatosis: case report and brief literature review. J Am Board Fam Pract 2003;16:555–9.

6. Nolle B, Specs U, Ludemann J, Rohrbach MS, DeRemee RA, Gross WL. Anticytoplasmic autoantibodies: their immunodiagnostic value in Wegener's Granulomatosis. Ann Intern Med 1989;111:28-40.

7. Schonermarck U, Lamprecht P, Csernok E, Gross WL. Prevalence and spectrum of rheumatic diseases associated with proteinase 3-antineutrophil cytoplasmic antibodies (ANCA) and myeloperoxidase-ANCA. Rheumatology 2001;40:178-84.

8. Frankel SK, Cosgrove GP, Fischer A et al. Update in the diagnosis and management of pulmonary vasculitis. Chest 2006;29:452-65.

9. Frankel SK, Sullivan EJ, Brown KK. Vasculitis: Wegener granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis, polyarteritis nodosa, and Takayasu arteritis. Crit Care Clin 2002;18:855-79.

10. Cordier J-F, Valeyre D, Guillevin L et al. Pulmonary Wegener's granulomatosis: a clinical and imaging study of 77 cases. Chest 1990;97:906-12.

11. Anderson G, Coles ET, Crane M et al. Wegener's granulomatosis: a series of 265 British cases seen between 1975 and 1985: a report by a sub-committee of the British Thoracic Society Research Committee. Q J Med 1992;83:427-38.

12. Hoffman GS, Kerr GS, Leavitt RY et al. Wegener granulomatosis: an analysis of 158 patients. Ann Intern Med 1992;116:488–98.

13. Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. Ann Intern Med 1983;98:76–85.

14. Thickett DR, Richter AG, Nathani N et al. Pulmonary manifestations of anti-neutrophil cytoplasmic antibody (ANCA)-positive vasculitis. Rheumatology 2006;45:261-8.

15. Schnabel A, Reuter M, Csernok E et al. Subclinical alveolar bleeding in pulmonary vasculitides: correlation with indices of disease activity. Eur Respir J 1999;14:118-24.

16. Bligny D, Mahr A, Toumelin PL et al. Predicting mortality in systemic Wegener's granulomatosis: a survival analysis based on 93 patients. Arthritis Rheum 2004;51:83-91.

17. Neuman I, Kain R, Regele H et al. Histological and clinical predictors of early and late renal outcome in ANCA-associated vasculitis. Nephrol Dial Transplant 2005;20:96-104.

18. Gayraud M, Guillevin L, le Toumelin P et al. Long-term follow up of polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: analysis of four prospective trials including 278 patients. Arthritis Rheum 2001;44:666-75.

19. Courthaliac C, Aumaıˆtre O, Andre´ M et al. Apport de la tomodensitome´ trie dans le suivi e´ volutif des le´ sions pleuropulmonaires de la granulomatose de Wegener. Rev Med Interne 1999;20:571–8.

20. Guillevin L, Cordier J-F, Lhote F et al. A prospective multicenter randomized trial comparing steroids and pulse cyclophosphamide versus steroids and oral cyclophosphamide in the treatment of generalized Wegener's granulomatosis. Arthritis Rheum 1997;40:2187–98.

21. Haubitz M, Schellong S, Gobel U et al. Intravenous pulse administration of cyclophosphamide versus daily oral treatment in patients with antineutrophil cytoplasmic antibody-associated vasculitis and renal involvement: a prospective randomized study. Arthritis Rheum 1998;41:1835–44.

22. de Groot K, Adu D, Savage CO. The value of pulse cyclophosphamide in ANCA-associated vasculitis: meta-analysis and critical review. Nephrol Dial Transplant 2001;16:2018–27.

23. Langford CA, Sneller MC, Hoffman GS. Methotrexate use in systemic vasculitis. Rheum Dis Clin North Am 1997;23:841–53.

24. Langford CA, Talar-Williams C, Sneller MC. Use of methotrexate and glucocorticoids in the treatment of Wegener's granulomatosis. Long-term renal outcome in patients with glomerolonephritis. Arthritis Rheum 2000;43:1836–40.

25. Papiris SA, Manoussakis MN, Drosos AA, Kontogiannis D, Constantopoulos SH, Moutsopoulos HM. Imaging of thoracic Wegener's granulomatosis: the tomographic appearance. Am J Med 1992;93:529-36.

NON-HODGKIN'S LYMPHOMA PRESENTING AS A PRIMARY ENDOBRONCHIAL TUMOR- A CASE REPORT

Goel KM, K Pritpal, D Sean, Ajay K, Chaudhary SK, Karan Madan

Pulmonology Department, Delhi Heart and Lung Institute, New Delhi, India

ABSTRACT

Non-Hodgkin's lymphoma (NHL) involving the endobronchial tree is uncommon, and the initial presentation of NHL as an endobronchial tumor is extremely rare. Several clinical reports have described bronchial-associated lymphoid tissue (BALT) lymphoma as an endobronchial lesion.

A 77 year old male hospitalized in another hospital for acute breathlessness and mechanically ventilated. He was shifted to Delhi Heart And Lung Institute because of failed extubation after 3 days of mechanical ventilation and reintubated .Past History of intubation was present 1 month back and diagnosed as a case of acute bronchitis. On evaluation at another hospital, the patient was found to have normal chest radiograph. Chest examination revealed findings consistent assisted ventilatory breath sounds associated with bilateral ronchi. Blood investigations were within normal limit. Contrast enhanced computed tomography of the chest revealed endoluminal soft tissue mass lesion at carina significantly obliterating bilateral main bronchi.USG Whole Abdomen revealed mild hepatomegaly and left renal cortical cyst measuring 4×5 cm and grade I BPH.Fibreoptic bronchoscopy revealed a globular smooth mass causing near complete obstruction of left main bronchus. Histopathological examination of the endobronchial biopsy showed tumor cells have a round or oval nucleus that appears vesicular because of margination of chromatin at the nuclear membrane, but large multilobed or cleaved nuclei predominate in some cases . Immunohistochemical staining was positive for LCA, CD20, and CD79a and negative for CD3, CD5, CD30, NSE, CK, Ki67, Chromogranin and Synaptophysin. While in the hospital, the patient was managed with mechanical ventilation and symptomatic treatment. FOB and rigid Bronchoscope, debulking of tumour growths was done using electrocautery snare. Patient was continued on overnight mechanical ventilation and extubated after one day. Post extubation, patient remained alright without any respiratory distress and discharged in stable condition. Latter on patient followed in Rajiv Gandhi Cancer Hospital. He underwent PET scan of whole body, which revealed normal study. Patient was managed with chemotherapeutic agents and he is still alive after 3 years of management without any symptoms.

NHL rarely presents as an endobronchial growth and only histopathology can differentiate it from other benign and malignant endobronchial masses.

Key words: Non Hodkin Lymphoma, Primary Edotracheal Tumour

INTRODUCTION

Non-Hodgkin's lymphoma (NHL) involving the endobronchial tree is uncommon.[13] It is rare for an endobronchial lesion to be the primary presentation of lymphoma, comprising <1%of all NHL. Endobronchial lymphoma is classified into two types, according to pattern of involvement. Type 1 includes diffuse submucosal infiltrates originating from hematogenous or lymphangitic spread in the presence of systemic lymphoma. Type II (similar to our patient) includes airway involvement by a localized mass due to direct spread of lymphoma from adjacent lymph nodes or arising de novo from bronchus-associated lymphoid tissue (BALT). The differential diagnosis of BALT lymphoma includes reactive lymphoid conditions such as

Correspondence:
Dr Manoj K Goel
Director, Delhi Heart & Lung Institute
3MM-II Panchkuian Road, New Delhi -110055
Tel: 011-42999999 EXN: 6054, Fax: 011-23514489
E-mail: drgoelmanoj@yahoo.co.in

intrapulmonary lymph node, lymphoid hyperplasia, follicular bronchitis and interstitial lymphoid hyperplasia. Overall, the 5-year survival is over 80% and the median survival time is greater than 10 years.[11]

Case Summary

A 77 year old male hospitalized in another hospital for acute breathlessness and mechanically ventilated. He was shifted to Delhi Heart And Lung Institute because of failed extubation after 3 days of mechanical ventilation and reintubated. Past History of intubation was present 1 month back and diagnosed as a case of acute bronchitis He was conscious, oriented comprehending verbal commands. On evaluation at another hospital, the patient was found to have normal chest radiograph. At admission, the patient was afebrile, had endotracheal tube in situ, heart rate 109/min and a respiratory rate18 /min. There was no pallor, clubbing, lymphadenopathy or pedal oedema. Chest examination revealed findings consistent assisted ventilatory breath sounds associated with bilateral ronchi. The rest of systemic examination was unremarkable. On investigation, hemoglobin, arterial blood gases levels, blood counts and metabolic parameters were within normal limits**.** Sputum smear examination by Gram's and Ziehl-Neelsen's stains was negative**.**

Chest radiograph showed normal study (Figure 1). Contrast enhanced computed tomography of the chest revealed endoluminal soft tissue mass lesion at carina significantly obliterating bilateral main bronchi (Figure 2, 3). USG Whole Abdomen revealed mild hepatomegaly and left renal cortical cyst measuring 4×5 cm and grade I BPH.Fibreoptic bronchoscopy revealed a globular smooth mass causing near complete obstruction of left main bronchus (Figure 4.)

Histopathological examination of the endobronchial biopsy showed tumor cells have a round or oval nucleus that appears vesicular because of margination of chromatin at the nuclear membrane, but large multilobed or cleaved nuclei predominate in some cases(Figure 5) .Nucleoli may be 2-3 in number and located adjacent to the nuclear membrane, or they may be single and centrally placed. Cytoplasm is usually present in moderate abundance and may be pale or basophilic. Immunohistochemical staining was positive for LCA, CD20, and CD79a and negative for CD3, CD5, CD30, NSE, CK, Ki67, Chromogranin and Synaptophysin. While in the hospital, the patient was managed with mechanical ventilation and symptomatic treatment. FOB and rigid Bronchoscope, debulking of tumour growths was done using electrocautery snare. The patient had mild bleeding which could be controlled easily. Airway patency on both sides was restored.Patient was continued on overnight mechanical ventilation and extubated after one day. Post extubation, patient remained alright without any respiratory distress and discharged in stable condition. Latter on patient followed in Rajiv Gandhi Cancer Hospital. He underwent PET scan of whole body, which revealed normal study. Patient was managed with chemotherapy in the form of dexamethasone, cyclophosphamide, doxorubicin, vincristine and etoposide. The patient showed good response.He is still alive after 3 years of management without any symptoms and again followed in our hospital.

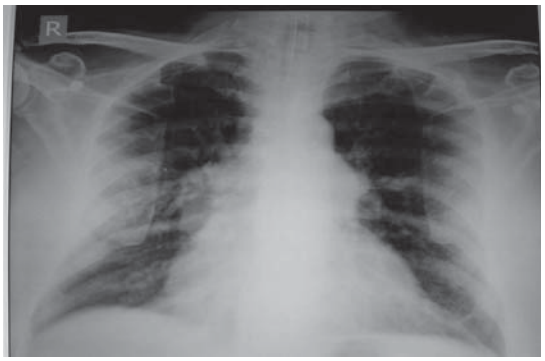


Figure 1: Chest radiograph

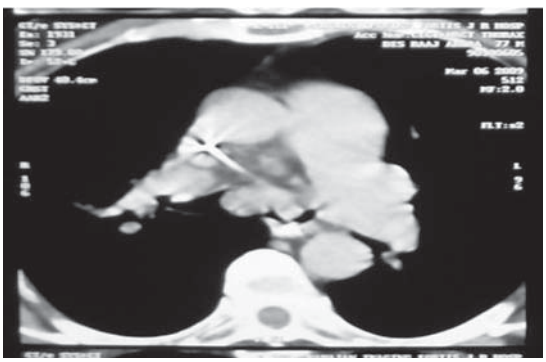


Figure 2: Computed tomographic scan of the chest showing endoluminal soft tissue mass lesion at carina significantly obliterating bilateral main bronchi

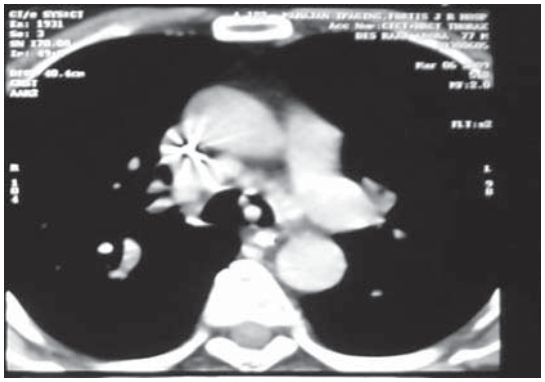


Figure 3: Computed tomographic scan of the chest showing endoluminal soft tissue mass lesion at carina significantly obliterating bilateral main bronchi

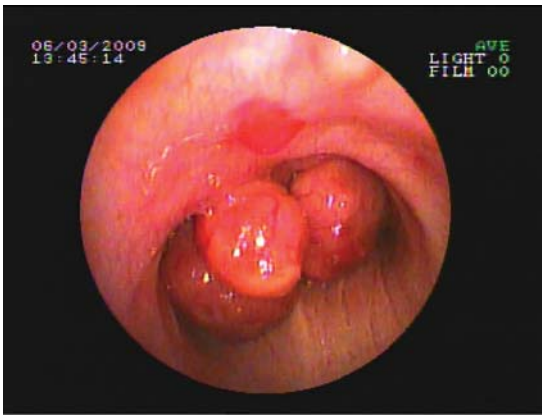


Figure 4: Bronchoscopy showing multiple pedunculated growths seen just above carina almost fully obscuring the lumens of both main bronchi

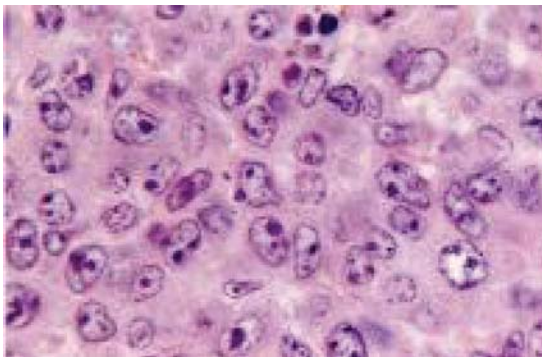


Figure 5: Diffuse large B-cell non-Hodgkin lymphoma. Large cells with abundant cytoplasm and large round-ovoid nuclei with thick nuclear membrane and multiple prominent nucleoli

DISCUSSION

The first case of endobronchial NHL was described in 1955 by Dawe *et al.*[4] Since then, about 50 cases have been described in the literature.[2,3] In an autopsy study of patients with NHL (n=55), where none of the cases showed endobronchial involvement.[14] In another autopsy study, only one patient had endobronchial lesion out of 93 patients with pulmonary lymphoma.[10] These observations suggest that endobronchial involvement is very rare in patients with NHL. The involvement of tracheobronchial tree is more common in Hodgkin's lymphoma than in NHL. The most common involvement is displacement or narrowing of airway lumen by enlarged mediastinal or hilar lymph nodes[12], followed by diffuse peribronchial infiltrates resembling lymphangitis carcinomatosis[7] and as an endobronchial mass like in the present case. The endobronchial lesion can be solitary[6,12] or multiple.[2,5] The symptoms depend upon the type of involvement of intrathoracic sites and the morphological subtype of NHL. The anaplastic large cell lymphoma accounts for about 2% of all NHLs.[1] The most common extanodal site of occurrence of this type of lymphoma is the skin and its primary occurrence at other extranodal sites is rare.[3] Chest radiograph shows the features of atelectasis or obstructive pneumonitis in most of the cases with endobronchial involvement. There are different mechanisms postulated for the development of endobronchial lesions in lymphoma. These include direct invasion from adjacent mediastinal or parenchymal disease, lymphatic spread to peribronchial connective tissues or hematogenous spread.[14] In the present case, there was only primary endobronchial involvement not due to extension from the mediastinal lymph node. Bronchoscopy and endobronchial biopsy is the definitive investigation. Treatment depends on the extent of involvement of the tumour and the general condition of the patient. Most of the patients are managed with chemotherapy and/ or radiotherapy. The 5-year survival is over 80% and the median survival time is greater than 10 years. Poor prognostic indicators include age over 60 years, elevated B microglobulin, the lack of t(11,18)(q21,q21), persistent of disease after completion of chemotherapy.[11]

CONCLUSION

NHL rarely presents as an endobronchial growth and only histopathology can differentiate it from other benign and malignant endobronchial masses.

REFERENCES

1. Armitage JO, Longo DL. Malignancies of lymphoid cells. *Harrison's Principles of Internal Medicine* New York: McGraw-Hill. 2001;725.
2. Banks DE, Castellan RM, Hendrick DJ. Lymphocytic lymphoma recurring in multiple endobronchial sites. *Thorax* 1980;35:796-7.
3. Bhalla R, McClure S. Pathological quiz case: a 17-year-old adolescent girl with a short history of dyspnea. Endobronchial, anaplastic large cell lymphoma, T-cell phenotype. *Arch Pahtol Lab Med* 2003;127:430-1.
4. Dawe CJ, Woolner LB, Parkhil EM, McDonald JR. Cytological studies of sputum secretions and serous fluids in malignant lymphoma. *Am J Clin Pathol* 1955;25:480-8.
5. Ieki R, Goto H, Kouzia Y, Morisaki T, Asano S,Shimada K, *et al.* Endobronchial non-Hodgkin's lymphoma. *Respir Med* 1989; 83: 87-9.
6. Kilgore TL, Chasen MH. Endobronchial non-Hodgkin's lymphoma. *Chest* 1983;84:58-61.
7. Lee KS, Kim Y, Primack SL. Imaging of pulmonary lymphomas. *Am J Roentgenol* 1997;168:339-45.
8. McRae WM, Wong CS, Jeffery GM. Endobronchial non-Hodgkin's Lymphoma. *Respir Med* 1998;92:975-7.
9. Ogawa Y, Takada Y, Okada S, *et al.* Four cases of malignant lymphoma with intrabronchial involvement. *Jpn J Chest Dis* 1981;44:699-704.
10. Papaioannou AN, Watson WL. Primary lymphoma of the lung: an appraisal of its natural history and a comparison with other localized lymphomas. *J Thorac Cardiovasc Surg* 1965;49:373-87.
11. Samuels ML, Howe CD, Dodd GD Jr, Fuller ML, Shullenberger CC, Leary WL. Endobronchial malignant lymphoma. Report of five cases in adults. *Am J Roentgenol Radium Ther Nuclear Med* 1961;85:87-90.
12 P. Kavoura, S. Kalampoka, H. Lambrakis, G. Birba, D. Amaxilati, L. Papageorgiou, P. Roussou, F. Vlastos. Balt lymphoma, a rare endobronchial tumor. A case Report. 19th ERS Annual Congress - September 12-16, 2009;2716
13. Soloman A, Zuckerman T, Gerenuk L, Ben-Arieh Y, Rowe JM, Yigla M. Non-Hodgkin Lymphoma presenting as an endobronchial tumour: report of eight cases and literature review. Am J Hematol. 2008; 83:416-9.
14. Vieta JO, Cravor LF. Intrathoracic manifestations of the lymphomatoid diseases. *Radiology* 1941;37:138-59.

SAARC Tuberculosis and HIV/AIDS Centre (STAC)
Thimi, Bhaktapur
G.P.O. Box 9517, Kathmandu, Nepal
Tel.: 00977-01-6632601, 6632477, 6631048
Fax: 00977-1-6634379
E-mail: saarctb@mos.com.np
Website: www.saarctb.org