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EDITORIAL

Original Articles

Case study

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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.l.

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Editorial

Poverty and HIV/AIDS

HIV/AIDS is not simply a health issue and cannot be addressed through the health sector alone. Globally, an estimated 35.3 million people were living with HIV in 2012. HIV epidemic in SAARC region is continued to be a major public health problem. All eight Member States of the SAARC region are designated as low prevalence countries. However, as per latest available information this region is home for an estimated number of 2.24 million HIV infected people.

In SAARC Region the first HIV infected persons were diagnosed in 1986 in India and Pakistan. By 1993, all the SAARC Member States had reported the existence of HIV infection in their countries. After more than 25 years of struggle against HIV, the SAARC Region is witnessing unprecedented returns from its investment in HIV prevention, treatment and care. The annual number of new HIV infection has declined. Effective ART has been demonstrated by significant reduction in the rate of ongoing HIV transmission. Despite this progress in global and regional response, economic inequality, social marginalization and other structural factors continue to fuel the HIV epidemic with estimated 1.56 lakh dying from AIDS in year 2012 in SAARC region. In the region HIV/AIDS is increasingly concentrated among persons marginalized by poverty, inequities and stigma. Social, economic and legal disadvantages increase vulnerability to HIV.

The impact of infection is already evident on health care systems e.g. decreasing life expectancy figures. The economic negative impact of HIV is immense, particularly among poor individuals and households. While all chronic diseases take their toll on human productivity, HIV is markedly worse because it affects people in their prime productive years of 15-49 years of age.

Many studies have documented the impact on families and communities, particularly due to the loss of income from both the infected persons and those who care for them, expenditures on health care and funerals, loss of future earnings and investment as children drop out of school to earn money or help caretakers, decreased agricultural production due to lack of labor, lost savings, lost homes, land sold to cover health costs and increased indebtedness.

Many developing countries are focusing on poverty reduction as a key component of their overall development. Poverty reduction strategies are now a key to all government in SAARC region. HIV/AIDS clearly has an impact on the prospects of poor people who become infected with the virus and, as such, it needs to be given careful consideration in any poverty reduction strategy.

Data on the extent of this problem proves elusive. However, the most striking figure is simple to understand. The 95 percent of those infected with HIV live in developing countries, home to around 85 per cent of the world's population. There is a strong positive association between HIV globally and the absolute poverty rate (at both $1 and $2 a day levels).

Some research suggest that investment in ART for maintaining 3.5 million people on treatment would save 18.5 million life-years and return US$ 12-34 billion mainly through increased labour productivity and averted orphan care over 2011-30. A recent systematic review of the literature on economic and quality of life outcomes of ART for HIV/AIDS in developing countries concluded that ending AIDS would lead to better skilled, productive workforce and stronger economies. Special commission on health convened by The Lancet concluded that additional life years due to health improvements were responsible for 24% of the growth in income in low and middle income countries from 2000-2011.

So it is high time to re-think about the control and prevention of HIV, which cannot be addressed solely through medical and clinical approaches, but through combined efforts to work across sectors to avoid working in isolation. The region needs to leverage capacity to build a new movement to achieve common goals to end AIDS and to end extreme poverty by 2030.

A COMPARISION OF LABORATORY DIAGNOSTIC METHODS OF TUBERCULOSIS AND AETIOLOGY OF SUSPECTED CASES OF PULMONARY TUBERCULOSIS

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ABSTRACT

Introduction: Tuberculosis remains a worldwide public health problem despite the highly effective drugs and vaccines are available making tuberculosis a preventable and curable disease. The objective of this study was to compare the different laboratory diagnostic methods of tuberculosis and determine its prevalence.

Methodology: Morning sputum samples were collected from suspected cases of pulmonary tuberculosis and proceeded for Ziehl Neelsen staining, fluorescent staining (auramin-O) and mycobacterium culture in Lowenstein Jensen medium.

Results: Total 78 suspected cases of pulmonary tuberculosis were included in this study among them 53 were male and 25 were female. Out of 78 cases, 46 cases were found to be culture positive. In culture positive cases (83%) were found to be *M. tuberculosis* and (17%) were found to be slow grower, fine colonies, AFB positive but niacin test negative (mycobacteria other than *M. tuberculosis*). In the direct microscopic examination by Ziehl Neelsen stained smear 26 samples were found to be acid fast bacilli and one sample was culture negative but acid fast bacilli positive. In fluorescent stained smear 34 samples were found to be positive for acid fast bacilli and 5 samples were culture negative but acid fast bacilli positive. Culture was accepted as gold standard, the sensitivity of direct microscopic examination was found 56.5% for Ziehl Neelsen staining and 73.9% for fluorescent staining respectively.

Conclusion: In culture positive cases *M. tuberculosis* and mycobacteria other than *M. tuberculosis* was found to be 83% and 17% respectively, it was found higher in male than female. Fluorescent microscopy is superior to Ziehl Neelsen microscopy but gives more false positive result than Z-N staining. Combining of Ziehl Neelsen and fluorescent staining is better than fluorescent staining alone.

Key words: Fluorescent Microscope, Mycobacterium Culture, *M. tuberculosis,* Ziehl Neelsen Stain.

INTRODUCTION

Tuberculosis is a disease of poverty affecting mostly young adults in their most productive years. The vast majority of TB deaths are in the developing world. The tuberculosis continues to be a great public health problem in Nepal. The number of new cases is increasing due to failure in early detection and drug resistance. These are the important problem in treatment and cure of the tuberculosis.[1] Cure of the disease is possible only with correct diagnosis and appropriate treatment. Early and accurate detection of active cases remains an important objective for improved implementation of chemotherapy and for reduction in the spread of the disease.[2] The diagnosis of tuberculosis is largely based on conventional approaches, which rely on clinical features and the results of X-ray, microscopy and culture examination. ZN staining or fluorescent staining allows highly accurate diagnoses which are widely available, simple and quick method.[3] The

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diagnosis traditionally depends upon identifying the infective organisms in secretions or tissues of diseased individuals. Sputum is the main sample for pulmonary tuberculosis.[4]

Isolation of organisms is the only definitive currently available mean for the diagnosis of TB. It has specificity that approaches 100% and also permits susceptibility testing of the isolates. But due to the poor availability of the culturing facilities, the diagnosis is often delayed and majority of the cases are mismanaged by unwise use of anti-TB drugs and other antibiotics, which may also result in developing the resistance. Although AFB smears examination (Microscopy) is time honored and economical, but for this technique the yields requirement is between 5000 to 10,000 organisms per ml.[5] Conventional culture methods are sensitive and can detect 10-100 organisms per sample. However, culture methods are time consuming and take 6-8 weeks for the results. Furthermore, viable organisms are needed for culture.[6, 7]

Mycobacterium tuberculosis is the most important causative agent of tuberculosis (TB) while nontuberculous mycobacteria (NTM) may play a key role in etiology of TB-like syndromes.[8] Treatment of TB patients in most countries is based solely on the results of microscopic smear positivity. As such, all sputum smear positive diagnosed patients are indiscriminately placed on DOTS, the current international TB treatment strategy. The implication is that NTM is inappropriately managed with first-line anti-tuberculous drug worsening the patient's condition and raising the risk of drug resistance.[9, 10] Although it is known that most sputum smear positive patients are truly TB patients.[11] The continued increase in TB drug resistance raises the question on the impact of this indiscriminate use of TB drugs to treat all diagnosed sputum smear positive patients. In this study, the staining methods (Z-N and fluorescent staining) are compared and evaluated taking mycobacterium culture on Lowenstein- Jensen (LJ) medium as gold standard and determine the aetiology of suspected cases of pulmonary tuberculosis.

METHODOLOGY

A Prospective study was conducted at German Nepal Tuberculosis project; Kathmandu duration of the study was from July 2010 to December 2010. In this study 78 suspected cases of pulmonary tuberculosis were included.

Sample collection

Sputum specimen was collected in clean, sterile, leak-proof, wide-mouth containers. The processing of the samples was carried out in a bio-safety cabinet. The sample collected was evaluated in terms of its acceptability, proper labeling such as full name, age, sex, serial number of the patient, date of collection.

Microscopy

Morning sputum sample was collected, stained by Ziehl Neelsen staining and auramine-O fluorochrome method and observed under compound binocular microscope and fluorescent binocular microscope respectively.

Culture

For mycobacterium culture sputum sample were decontaminated and centrifuged by using 4% NaOH, according to modified Petroff method and inoculated into Lowenstein Jensen medium. Lowenstein-Jensen media were incubated at 37°C and left in the slanted position for 7 days to permit even distribution of the inoculum over the entire surface of the medium. The tubes are then placed upright and incubation at 37°C for 6-8 weeks.

Observation of colony morphology

The colonies of *M. tuberculosis* were rough, dry, 3-4mm in diameter, raised, and thick with wrinkled surface and an irregular thin margin. They were non-pigmented (off-white to faint buff), tenacious and not easily emulsified.

Identification of *M. tuberculosis*

For identification of *M. tuberculosis* rate of growth, colonies characters, AFB staining and niacin test were used as per manufacture instruction.

Niacin drop test

Niacin drop test was performed according to manufacturer instruction to identify *M. tuberculosis* and differentiated it from mycobacteria other than *M. tuberculosis.*

Ethical consideration

The research objective and methods were explained to the patient and informed consent was

taken from each participant before collection of sputum specimen.

Data analysis

Data was analyzed by EPI-Info version 3.3.2, document version 8.08 updated Sept 2005 and presented by chart and diagrams.

RESULTS

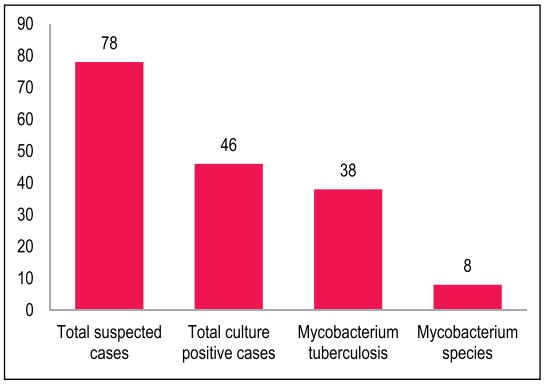

Figure 1. Total suspected and mycobacterium culture positive cases culture positive cases

Out of 46 cultures positive cases, 38 (83%) was found *M. tuberculosis* and 8 (17%) was found mycobacteria other than *M. tuberculosis.*

Forty six cases were found to be mycobacterium culture positive. In the direct microscopic examination by Ziehl Neelsen and fluorescent stained smear 26 and 34 samples were found to be acid fast bacilli and one and five samples were culture negative but acid fast bacilli positive respectively.

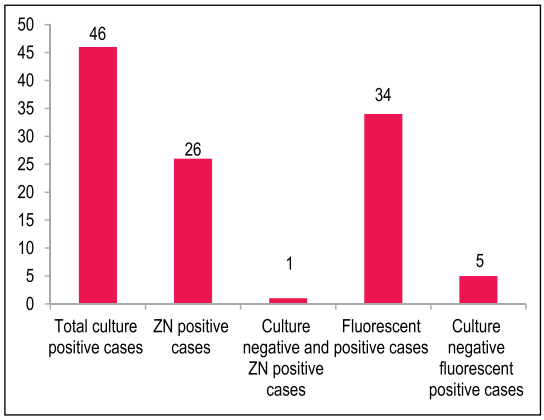

Figure 2. Comparison of mycobacterium culture, Ziehl Neelsen and fluorescent microscopy

Mycobacterium species was found higher in male than female and isolated maximum in age group 16-30 (43.8%) followed by the age group 46-60 (34.7%).

Niacin test was performed for 46 culture positive cases. Among them 38 was niacin positive (*M. tuberculosis*) and 8 was niacin negative (mycobacteria other than *M. tuberculosis*).

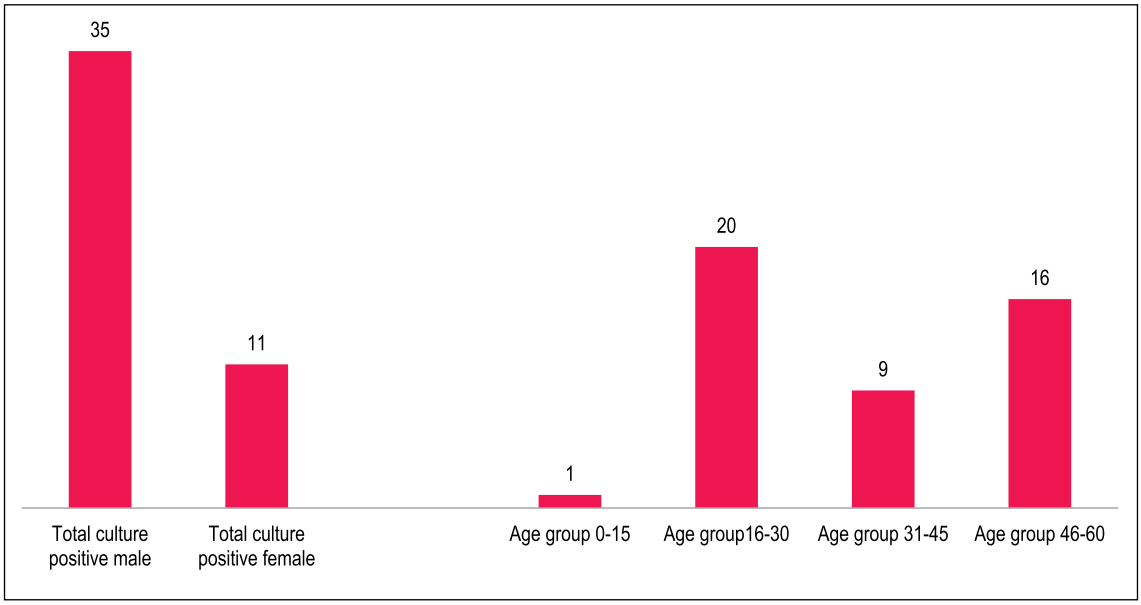

Figure 3. Gender and age wise distribution of positive cases

DISCUSSION

According to the WHO guidelines for TB control, patient with more than three weeks history of cough should be screened for pulmonary tuberculosis with direct sputum smear examination for *M. tuberculosis*. Because the clinical signs and symptoms of pulmonary tuberculosis are not specific, the fundamental principal for the diagnosis of tuberculosis is the accurate demonstration of *M. tuberculosis* in a suitable specimen from the suspected cases of pulmonary tuberculosis for the adequate treatment. Either presumptive diagnosis is based on the demonstration of tubercle bacilli in the sputum or *M. tuberculosis* may be demonstrated by culture.[12] Detection of smear positive cases is the highest priority in any TB control program, as these cases are infectious and contribute to transmission of disease. Though smear positivity correlates well with infectivity, much of the transmission occurs before the level of bacilli reach 10^5/ml in the sputum.[13] ZN stain can detect bacilli when they are in the order of 10^5/ml of the sputum whereas a more sensitive fluorescent stain can detect in the order of 10^4/ml of sputum.[14]

The findings of present study showed that out of 78 cases 46 (58.97%) cases was culture positive. In culture positive cases 83% was found to be *M. tuberculosis*. In culture positive cases 34 (73.9%) were positive for fluorescent staining (auramin-O) and 26 (56.5%) were positive for Ziehl Neelsen staining. This finding agrees with the study done in the other parts of the world fluorescent stain is superior to Ziehl Neelsen stain. A study conducted by Laifangbam *et al* showed that out of 102 patients suspected of pulmonary tuberculosis, 44.1%, 71.6% and 79% were found positive by ZN, auramine O staining and culture respectively.[13] Similar study conducted by Jain *et al* found sensitivity of ZN was 32.7% and auramine O staining was 41.6%[14], Githui *et al* found sensitivity of ZN was 65%, fluorescent staining was 80%[15], Ulukanligil *et al* found sensitivity of ZN was 67.6% and fluorescent staining was 85.7% [16] and Prasanthi and Kumar found sensitivity of ZN was 50% and fluorescent was 69%.[17] Fluorchrome stain is more efficient over ZN stain in detecting tubercle bacilli in sputum, especially the paucibacillary cases. Since screening is done under lower power of magnification (400X), fluorescent microscopy has been found to be less time consuming as compared to ZN method (1000X) in the diagnosis of tuberculosis. The fluorescing bacilli are easily identifiable and cause less eye strain. The advantages of sputum smear microscopy is that it has very close relation with infectiousness, patient who are sputum smear positive and culture positive are more likely to be infectious than culture positive but smear_negative. Thus the detection of AFB in sputum smear examination by microscopy play an important part in tuberculosis control programs as transmission of the disease is due mainly to patients whose sputum contains so many organisms that they are detectable by direct microscopy of sputum smear and is helpful to control highly transmission of tuberculosis patients. Ziehl Neelsen staining is rapid and inexpensive but requires a high amount of organisms in the specimens. Fluorescent microscopy is more significant than ZN microscopy but even has chance of observing false negative. Culture along with fluorescent microscopy should be the method of choice for the detection of TB cases in-spite of its time consuming demerit. The detection of TB cases on molecular level is also in practice in our country but due to the lack of molecular expertise, highly expensive equipment need and high test charges these techniques are not so common. Finding of this study suggested that at least fluorescent microscopy and culture must be recommended and should not be rely on clinical symptoms only for the treatment of tuberculosis cases.

Out of 46 culture positive cases 35 were male and 16 were female. In this study 76% of patients accounted male is higher incidence than the study conducted by Bhatt *et al* (64%)[18] and Bam (65%).[19] This finding agrees with the study conducted in National Tuberculosis Center Thimi, Bhaktapur, Nepal by Bhatt *et al*[20] it was found that the incidence of pulmonary tuberculosis was higher in male 75% than female 25%. In almost all areas where the TB is the public health problem, the incidence of TB among women is less than man. Gender is not merely the biological difference but the differences between men and women in their roles, behaviors, expectations and opportunities within a social cultural and economic context. Nepalese society encounters gender disparities profoundly in many aspects in their lives such as, education, job opportunities, food & nutrition, morbidity & mortality pattern of diseases and health care. The low status

accorded to women in male dominated country like Nepal, their limited decision making power, restricted mobility and poor access to health care resources make them particularly vulnerable to ill health and reduce opportunities in accessing basic and available health care.

The majority (67.3%) of patients belongs to economically active young age group 16-45 years. The proportion was nearly same as reported by Bhatt *et al*[18] (62.7%) of the respondents belong to 21-50 years age group and Bam[19,20] reported (95%) was 15-54 years age group. More than 90% of global TB cases and death occur in the developing world, where 75% of cases are within the economically most productive age group (15-54 years). An adult with TB (in the developing world) loses on average 3-4 months of work time and the economic losses to the family and community are staggering. The estimates suggest a loss of 20-30% of annual household income and, if the person dies of the disease, an average of 15 years of lost income.[21] The finding of this study showed that *M. tuberculosis* was found to be 83% and mycobacteria other than *M. tuberculosis* was 17%. Similar study conducted by Shanker *et al* found 7.9% were atypical mycobacterium.[22] Kumar and Khurana found incidence of *M. tuberculosis* and atypical mycobacteria 91.6% and 8.4% respectively.[23] Other study conducted by Bhatt *et al* found all the isolates were *M. tuberculosis.*[18] Mycobacteria have been isolated from many sources including soil, animal and human faces, marshland, water (including lakes, rivers, estuaries, swimming pools, aquaria, and domestic water supplies), vegetation and human skin.[24] Definitive diagnoses of pulmonary nontuberculous mycobacteria infection are difficult. Because the organisms are often saprophytes, they may colonize airways rather than infect them. Cultures can be falsely positive in patients with chronic lung disease and falsely negative in infected patients without cavities.[25] Thus, identification of acid-fast bacilli at microscopy or isolation of nontuberculous mycobacteria in culture by itself is not enough evidence for establishing the diagnosis.[26] Further there is cross-reactivity between MAC and *M. tuberculosis* on the purified protein derivative standard test.[27]

Sample size of this study is small and for identification of *M. tuberculosis* rate of growth, colonies characters, AFB staining and biochemical test only niacin test was performed. So that it was not possible to identify niacin negative mycobacterium species (mycobacteria other than *M. tuberculosis)*. It is therefore recommended that a large sample size that would cover rural and urban area of Nepal to compare different diagnostic methods, determine prevalence of *M. tuberculosis* and identification of mycobacterium species different biochemical tests should be considered in further investigation.

CONCLUSIONS

The findings of this study showed that the efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy. Combining of Ziehl Neelsen and fluorescent staining was better than fluorescent staining (auramine-O) alone both the staining significantly improve sensitivity and percentage of false negative result. In culture positive cases *M. tuberculosis* and mycobacteria other than *M. tuberculosis* was found to be 83% and 17% respectively, it was found higher in male than female. Diagnosis of pulmonary atypical mycobacterium infection may be difficult. The clinical and radiographic manifestations of infection are variable and frequently overlap. For the starting appropriate treatment of atypical mycobacterium diagnosis is the first step that assures full chances of cure because atypical mycobacterium are resistant to standard regime for tuberculosis.

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ASSESSING QUALITY OF LIFE AND DEPRESSION AMONG PEOPLE LIVING WITH HIV/AIDS AND TB-HIV COINFECTION IN KATHMANDU, NEPAL

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ABSTRACT

Introduction: Assessing the health related quality of life (HQoL) and depression in human immunodeficiency virus (HIV) positive people and TB-HIV coinfected people is of extreme importance in designing strategies and implementing interventional programs on treatment care and support to People living with HIV and AIDS.

Methodology: A cross-sectional study was conducted among People living with HIV/AIDS and TB-HIV Coinfection from July to December 2011 at care and support canters in Kathmandu, Nepal. The list HIV care and Support centre registered at Kathmandu were selected using the lottery proportionately to meet the sample size of 154. The method of data collection was summarized in the World Health Organization's questionnaire for Health related Quality of life and Beck Depression Inventory Scale II for depression. Data was collected using verbal information of respondents, entered on Epi Data version 3.4.1 and analyzed using SPSS version 16.0.

Results: TB-HIV coinfected people had lower quality of life in all domains compared to HIV/AIDS infected people. The prevalence of depression was found higher in TB-HIV coinfected people than HIV/AIDS group. CD4 count, educational status, occupation, ethnicity, family size and depression were statistically significant with the QoL domains. Among coinfected patients, depression was 3.86 times more likely to influence QoL while adjusted odds ratio was 4.21 times higher.

Conclusion: The TB control program should design strategies to improve the quality of life of TB-HIV coinfected people and depression should be targeted as an intervention to improve the quality of life of people living with HIV with or without TB.

Key words: Nepal, World Health Organization, Quality of Life Instrument, Beck Depression Inventory-II

INTRODUCTION

With the detection of Human Immunodeficiency Virus (HIV) for the first time in Nepal in 1988, HIV/AIDS is now increasingly considered as a chronic disease and is in the stage of a concentrated epidemic. Tuberculosis, more commonly known as TB, is a bacterial infection that usually affects the lungs but can affect any part of the body except hair, teeth and nails and is one of the main causes of HIV-related morbidity and mortality.[1] When a person is infected with HIV, they are at an increased risk of also contracting TB. Co-infection with TB can also mean an accelerated progression to AIDS. A HIV-negative person with a latent TB infection has a 10% chance of progressing to active TB over his or her entire lifetime, whereas a HIV-positive person has a 10% chance of developing active TB each year.[2] Many of the TB-HIV and HIV patients struggle with numerous social problems such as stigma, poverty, depression, substance abuse, and cultural beliefs which can affect their QoL not only from the physical health aspect, but also from a mental and social health point of view, and this causes numerous problems relating to the

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patients' useful activities and interests. Assessing health-related quality of life (HQoL) is useful for documenting the patients' perceived burden of chronic disease, tracking changes in health over time, assessing the effects of treatment and quantifying the return on health care investment. The World Health Organization (WHO) has defined quality of life "as an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns". According to WHO "Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. These problems can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her everyday responsibilities."The unparallel magnitude and scale of the HIV/ AIDS and TB epidemic remain major public health challenges globally.[3] Nepal has a concentrated HIV and AIDS epidemic, with an estimated 61,000 persons living with HIV and AIDS and about 14 new HIV infections each day.[4] The estimated prevalence of HIV among TB patients between the ages of 15-49 years and the estimated number of active TB cases among PLHIV in Nepal is 2.4%.[5] The assessment of QoL is central to understanding how people's lives are affected by TB and HIV infection. Optimization of HQoL is particularly important now that TB and HIV Coinfection can be considered a chronic disease with the prospect of long-term survival. The assessment of HQoL in this population can help us to detect problems that may influence the progression of the disease.

METHODS

Study Design

The study was cross sectional in nature and was prepared with the aid of quantitative data.The study was conducted at care and support centre i.e. Prerana, Navakiran Plus, Genetup, National Association of PLHA in Nepal (NAP+N) for 6 months starting from July 2011.

Study Population

The study population included people above 16 years including men and women with HIV/AIDS and TB-HIV Coinfection.

Inclusion and Exclusion Criteria

The study subjects above 16 years who had the history of HIV/AIDS and TB-HIV co-infection were included in the study. However the study subjects below 16 years and with auditory, visual and cognitive disability were not included in the study. Besides this, those who were unwilling to disclose the required information were excluded and unavailability of respondents was considered.

Sampling Method and size

The sample population was 154 including the 10% of non response rate at 95% confidence interval. The Random sampling technique followed by systematic selection of respondent was used by following procedure:

1. List of care and support centres present at Kathmandu valley were ranked anonymously and four care and support centres were chosen to meet the required sample size proportionately from the lottery.
2. Systematic sampling Method was done to select the respondents at this care and support centers.
3. During administration of questionnaire 50% of PLWHA were women.
4. Unavailability of respondents was addressed by keeping ten extra optionally selected samples at step 2.

Data Collection Procedure

The data was collected by interviewing the respondents using a structured questionnaire WHOQoL-HIV BREF[6] instrument consists of 31 items, with each item using a 5-point Likert scale and Beck Inventory Depression Scale II[7] which has 21 items.

Validity and Reliability

The study was conducted using the WHO health related quality of life indicator (HQoL) and Beck Inventory Depression Scale II which is valid, widely used and the most accepted instrument. Besides this, the pre testing was performed in order to check the acceptability of the questionnaire with the respondents. To measure internal consistency, the Cronbach's alpha was calculated for each domain of the instrument. Physical and Level of independence domains of WHOHQoL BREF questionnaire had a high value of Cronbach's alpha ($\alpha > 0.7$) i.e. 0.78 and 0.72. However, all of the other

remaining had an average value of Cronbach's alpha that is psychological health 0.68, social relationship 0.64, environmental 0.64, spiritual/ personal beliefs 0.66 and overall satisfaction 0.63.

Data Processing and Analysis

The data was entered in Epi data version 3.4.1 and analysis was done using SPSS version 16. Descriptive statistics for continuous data and frequencies & proportion for categorical data were calculated. For inferential statistics, t-test and one way ANOVA were used for bivariate analysis. Statistical significance was set at $p \leq 0.05$. Rank sum test and correlation were used for significant test and association. A binary logistic regression was performed to measure the strength of association between dependent and independent variables.

Ethical Consideration

The ethical approval was received from the Institutional Review Board of faculty of health science, Nobel College, Pokhara University. An informed verbal consent was ensured prior to administration of the questionnaire. The beneficence of the respondents was taken into consideration, subjects were not coerced to participate in the study and confidentiality was maintained. No incentives were provided for extraction of data. Cited references are presented in APA format for due acknowledgement of academic source and to avoid plagiarism.

RESULTS

Sample Characteristics

One hundred fifty four respondents were asked to participate in the study out of which 8 (6%) refused to participate in the study. The overall response rate of participation in the study was high, 146 (94%). The remaining participants refused to participate in the study explaining that they have participated in too many researches and few others said that they were not interested in our study.

Among all the participants 100%, 51.36% (n=75) were PLWHA and 48.63% (n=71) were coinfected with TB-HIV. Majority of respondents 55.48% (n=81) were males and were between the age of 26-35 years i.e.48.63 % (n=71) (table 1). 33.56 % (n=49) of the respondents had secondary level of schooling. As per the marital status 50 % (n=73) of the respondents were married followed by single, separated and divorced 1.54.11 % (n=79) of the respondents have CD4 count less than 350 other than 45.90 % (n=67) have CD4 count more than 350. Majority of the respondents i.e.68.50% (n=100) were employed and 56.16 % (n=82) lived in nuclear family (table 2).

The mean score for the six domains of HQoL and overall satisfaction regarding the general quality of life was highest in the environmental domain in both groups (22.08 and 24.08) followed by the psychological domain, i.e. 13.25 and 14.88, and the least in the physical and overall satisfaction domains. All the domains were found to have positive correlation with PLWHA and TB-HIV Coinfected people except the physical health domain in Coinfected people where no correlation was found.

The prevalence of depression among 71 TB-HIV Coinfected people and 75 PLWHA using beck depression scale was found to be 33.8% and 7.9% respectively (figure 1).

Depression and CD4 Count were associated with both the PLWHA and coinfected patients. Among coinfected patients, depression was 3.86 times more likely to influence QoL while, adjusted odds ratio was 4.21 times higher, OR = 4.211(95%CI: 1.22, 14.47). No significant association was observed with the sex, occupation and occupational status in both the groups (table 3).

Table 1. Quality of life of people with HIV/AIDS and TB-HIV Co infection

Quality of life domains	Number of items	TB-HIV Co-infection (n=71) Mean (SD)	Correlation with total score	HIV without TB/ HIV Co-infection (n=75) Mean (SD)	Correlation with total score	Proportion of respondents with QoL score	
						High	Low
Physical Health	4	11.99 (1.80)	0.041	11.52 (2.05)	0.385**	26.7	80.8
Psychological Health	5	13.25 (2.08)	0.515**	14.88 (2.24)	0.590**	37	89.7
Level of Independence	4	12.77(1.46)	0.406**	12.97 (1.77)	0.467**	42.5	86.3
Social Relationship	4	11.79 (2.47)	0.744**	13.36 (2.41)	0.680**	35.6	85.6
Environmental	8	22.08 (2.96)	0.773**	24.08 (2.71)	0.597**	26	80.1
Spiritual	4	11.08(2.48)	0.772**	13.09 (2.59)	0.725**	25.3	81.1
Overall satisfaction	2	5.25 (2.01)	0.783**	7.07(1.51)	0.507**	33.1	81.2

p*<0.05**p<0.001

Table 2. The Association of Socio-demographic Characteristics, quality of life, depression and disease severity among the people with HIV/AIDS and that with TB/HIV Co-infected (N=146)

Variables	Sample characteristics (%)	PH	Psy	Spri	Social	Env	Variables	Ind	Overall satisfaction
Sex							Sex		
Male	55.48	11.53	14.16	12.30	12.75	22.83	Male	13.00	6.00
Female	44.52	12.02	14.00	11.89	12.40	23.46	Female	13.00	6.00
T test		-1.501	0.416	0.890	0.827	-1.273	U test	2546.0	2604.5
Age									
16-25	17.80	11.88	13.96	12.12	12.38	23.73		12.96	6.38
26-35	48.63	11.66	14.10	11.90	12.59	23.28		12.97	6.24
36-45	28.09	11.80	14.10	12.46	12.78	22.37		12.59	5.93
46-55	5.48	12.25	14.38	12.45	12.38	22.38		13.25	6.38
F-test		0.353	0.067	0.370	0.147	1.320		0.679	0.356
Educational Status									
Primary	17.80	11.88	13.35	12.12	12.23	22.73		12.62	5.50
Lower secondary	6.16	12.44	15.00	12.22	13.11	23.56		12.56	6.56
Secondary		11.86	14.35	12.10	12.57	23.02		12.94	6.06
Above secondary	33.56	10.88	14.42	12.95	13.52	23.58		12.80	7.35
F-Test	27.38	3.083*	2.302	0.898	1.793	0.531		0.310	6.518**
Marital Status									
Married		11.79	14.07	12.56	13.03	23.07		12.67	6.01
Single	50.0	11.47	14.00	11.76	11.93	22.98		13.02	6.40
Separated	30.82	12.50	14.36	11.50	12.00	23.07		13.07	5.93
Divorced	9.59	11.64	14.21	11.57	13.07	23.79		13.29	6.64
F-Test	9.59	1.083	0.099	1.347	2.159	0.267		0.868	0.678
Current CD4 Count (cells/mm^3)									
≤350	45.90	11.85	13.07	11.09	11.69	21.82		12.64	5.10
>350	54.11	11.66	14.95	12.99	13.37	24.20		13.08	7.10
T-Test		.594	5.328**	-4.457**	-4.168**	5.192**		1.616	-6.978**
Occupation									
Employed	68.50	11.51	14.36	12.68	13.27	23.54		12.87	6.53
Unemployed	31.50	12.26	13.10	10.89	11.23	22.17		12.89	5.43
T-Test		2.193*	2.114*	3.856**	5.073**	2.607*		-.073	3.192*
Family Size							Family Size		
Nuclear	56.16	11.54	13.87	11.98	12.56	22.74	Nuclear	13.00	6.00
Joint	32.20	12.21	14.45	12.49	12.79	23.96	Joint	13.00	6.00
Single	11.64	11.47	14.18	11.76	12.24	22.53	Single	13.00	6.00
F Test		2.023	0.958	.688	.304	2.878*	Kruskal wallis test	0.559	3.400
Depression									
Minimal	Prevalence of depression is 41.7	11.58	14.58	12.82	13.24	23.59		13.03	6.58
Mild		12.80	13.30	11.90	11.90	22.90		12.70	5.20
Moderate		11.73	13.07	9.80	10.67	22.13		12.60	4.13
Severe		12.27	12.20	9.60	10.47	20.80		12.20	4.20
F Test		1.627	7.066**	12.748**	10.455**	4.781*		1.366	21.504**

*p<0.05, **p<0.001, PH = Physical health, Psy = psychological health, Social = Social relationship, Env = Environment, Ind = level of independence, Spir = Spiritual health

Table 3. Bi-variate and Multivariate Logistic Regression to measure the relationship of Independent variables with dependent variable

Variables	TB/ HIV Co-infection (n=71)		Variables	HIV/AIDS without TB (n=75)	
	Crude Odds ratio (95% CI)	Adjusted Odds ratio (95% CI)		Crude Odds ratio (95% CI)	Adjusted Odds ratio (95% CI)
Sex	0.955 (0.368-2.473)	0.995 (0.328-3.021)	**Sex**	1.458 (0.587-3.642)	1.322 (0.457-3.830)
Depression	3.860* (1.440-10.345)	4.211* (1.225-14.478)	**Depression**	2.259 (0.896-5.698)	1.858 (0.686-5.026)
Occupation	0.410 (0.156-1.080)	0.756 (0.238-2.398)	**Occupation**	0.411 (0.127-1.328)	0.475 (0.126-1.791)
CD4 Count	5.042* (1.268-20.053)	0.188* (0.043-0.818)	**CD4 Count**	0.095* (0.011-0.796)	0.103* (0.012-0.891)
Educational status	1.381 (0.425-4.490)	0.666 (0.150-2.961)	**Educational status**	2.000 (0.461-8.677)	2.044 (0.419-9.960)

*p<0.05

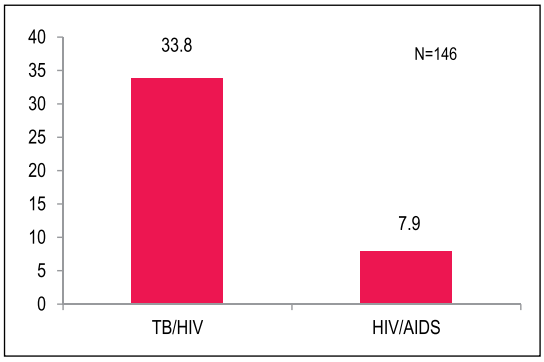

Figure 1. Prevalence of depression among PLHIV and TB/ HIV co-infection

DISCUSSION

In this study, comparison of the QoL of persons with HIV infection with and without TB was done. The WHOHQoL BREF questionnaire had a good internal consistency to assess the QoL of TB-HIV coinfected patients which was similar to the study conducted in Taiwan and Iran.[8,9] The overall response rate in the study was ninety-four percent which was similar to the study conducted in Croatia and Ethiopia where the response rate was above eighty percent.[10,11]

According to this study, the largest portion of respondents were affected with this disease between 18- 40 years of age and this finding is congruent the report of UNAIDS/WHO Report on the global AIDS epidemic 2008, in 2007 off total about 33 million people of HIV positive, an Estimates 30.8 million people were in the working age group (15-49 years of age). The majority of the respondents were males and this is similar to the research conducted in Bangladesh and Iran.[12,13] With more urban areas respondents were found to receive levels of secondary education similar to the other study.[13] Most of the respondents were married and were employed, in contrast with the other studies where most of the respondents were unemployed and separated. A majority of the respondents had a history of opportunistic infection and had CD4 Count more than 350 for PLWHA which was found to be similar to a study conducted in Bangladesh.[12]

The mean score of HQoL was highest in the environmental domain in both groups and the least in physical and overall satisfaction domains in this study, findings similar to the study in Bangladesh[12], where the mean QoL scores was highest in spiritual/ religion and lowest in the overall satisfaction, but in contrast with the study at Croatia.[10] TB-HIV co-infected patients had a lower quality of life in all domains as compared to HIV infected patients without TB.[11] In other studies, it was reported that HIV patients had a lower QoL as compared to the general population[14] and that TB patients had a lower QoL as compared to their neighbors.[15]

The Bivariate Analysis revealed that CD4 count, educational status, occupation, ethnicity, family size and depression was significantly associated with the QoL domains in the study while an association between CD4 count, WHO staging and other Socio demographic characteristics with QoL was observed in various studies.[15,16] Similarly,

Socio-demographic characteristics, such as age, gender, education, income employment status and disease related variables such as disease stage, opportunistic infection, CD4 count etc have been found to be strongly associated with the QoL of PLHIV.[17] Some researchers have documented low performance for women in some aspects of quality of life[18] while in some other studies difference according to gender was not found, or have even shown the opposite. Women had poorer QoL compared to men.[13] Some reports in literature find younger age to be related with better QoL[19] while in this study gender and sex has no relationship with the quality of life, which is similar to a study conducted at India.[20] Comparison with the educational status revealed that those with lower secondary and secondary education had better physical health and overall satisfaction whereas contradictory results have been observed in comparisons between educational groups which showed that those with less education reported significantly poorer QoL than those with more education, according to some authors. Likewise, Subjects with higher education reported better QoL in the independence and environment domains.[21] In this study marital status had no influence on the quality of life domains but marital status influences quality of life; Subjects who are married or in relationship reported a higher level of QoL for the social relationships domain. Those in relationships may enjoy better social support.[10] Having an occupation or a source of income meant a higher QoL in all the domains except level of independence which was similar to the results of study conducted in Brazil where source of income was associated with a better HQoL only with respect to the physical and psychological domains. Some authors have acknowledged the fact that being employed is the main predictor of the HQoL scales for their population and it has been shown that the HIV-infected patients find it difficult to get and keep jobs.[22] An association between CD4 count and other Socio demographic characteristics with QoL was observed in this study, in line with various other studies.[16,15] Studies show that depression affects the quality of life of people living with HIV in many ways,[23] which coincides with this study where depression had significant association with all the QoL domains except physical health and level of independence.

The prevalence of depression was higher in TB-HIV coinfected patients than PLWHA, findings which are similar to the results of a study where TB-HIV coinfected patients had significantly greater risk of CMD than the non-co-infected patients.[24] Clinical Management of TB-HIV co-infection can be complicated and due to the fact that many ART drugs pharmacokinetics and dynamics leads to drug interaction and predisposal symptoms, negative health effects and illness, as HIV leading to weak immune response itself is a risk factor for Neuro-psychiatric manifestations.[24,25] Variations in clinical outcomes of neuropsychiatric manifestations are observed in TB-HIV co-infections and HIV infection only. HIV only people are more symptomatic than in people with TB-HIV co-infection however these manifestations vary with the severity and viral load in the person.[26]

In coinfected patients, individuals who had depression were 8.8 times more likely to have poor physical health as compared to individuals who had no depression[11] whereas in this study, among coinfected patients, depression was 3.86 times more likely to influence QoL. When adjusted for the effect of potential confounding variables, the odds of having CMD for TB-HIV coinfected individuals was 1.7 times the odds for non-coinfected patient, while in this study adjusted odds was 4.21 times higher.[24]

CONCLUSION

TB-HIV co-infected patients had a lower quality of life in all domains compared to HIV infected patients without TB. The prevalence of depression was found higher in TB-HIV coinfected people than HIV/AIDS group. The Bivariate Analysis revealed that CD4 count, educational status, occupation, family size and depression were significantly associated with the QoL domains in the study. Gender, sex and marital status had no relationship with the quality of life. A significant association between CD4 counts and depression with QoL was observed with all the domains except physical health and level of independence. Among coinfected patients, depression was 3.86 times more likely to influence QoL,4.21 times higher with adjusted odds. The TB control program should design strategies to improve the quality of life of TB-HIV coinfected

people and depression should be targeted as an intervention to improve the quality of life of people living with HIV with or without TB.

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COMPARATIVE EVALUATION OF BACT/ALERT 3D CULTURE MEDIUM AND NESTED PCR IN THE DIAGNOSIS OF TUBERCULOUS MENINGITIS

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ABSTRACT

Introduction: We evaluated the efficacy of BacT/ALERT 3D culture medium in comparison with nested polymerase chain reaction (PCR) for early diagnosis of *Mycobacterium tuberculosis (MTb)* in cerebrospinal fluid (CSF).

Methodology: A total of 50 clinically suspected patients with CSF findings suggestive of TBM (Tuberculous meningitis) and 20 healthy controls were included in the present study.CSF from each of the patient was inoculated in BacT/ALERT-MP culture media along with the culture on convention Lowenstein Jensen's medium. Simultaneously nested PCR was carried out both in CSF and growth positive above culture media targeting heat shock protein gene *(hsp65),* a conserved gene of *MTb.*

Results: The culture showed 76% (38/50) positivity in TBM; however, 15% (3/20) of controls also showed false positivity by BacT/ALERT-MP system. By nested CSF-PCR, TBM could be detected in 92% (46/50) cases and 5% (1/20) in the controls. The other interesting observation was that when DNA isolated from broth of BacT/ALERT MP culture were subjected for PCR amplification, 15 (39.5%) of them yielded the desired amplicon for *MTb* while the broths subjected to conventional LJ media yielded for *M. tuberculosis* in 12of the 38 bottles (31.5%). None of culture negative medium was positive by PCR. Sensitivity and specificity of BacT/ALERT MP culture considering all of them as real positive were 76% and 85% while for nested PCR the corresponding values were 92% and 95% respectively.

Conclusions: BacT/ALERT 3D culture medium seems to be unsatisfactory as isolation sensitivity rate is very poor. Moreover, growth indicated in the medium must be confirmed for being Mycobacteria as other contaminants can also grow.

Key words: *Mycobacterium tuberculosis,* Tuberculous meningitis, Bact/ALERT, CSF PCR.

INTRODUCTION

WHO has estimated the incidence of new cases of active tuberculosis to be 139/100,000 population annually worldwide with ~1.6 million deaths per year.[1] Central nervous system (CNS) tuberculosis is one of the severest forms of the disease resulting into high morbidity, serious neurological defects and mortality.[2] In the recent past, number of immunocompromised population has increased due to high prevalence of HIV/AIDS, increasing incidence of diabetes mellitus, growing geriatric population and increased use of immunosuppressive drugs. Tuberculous meningitis (TBM) is rampant due to above mentioned reasons.[1] For better prognosis and minimum neurological sequels, an accurate and prompt diagnosis is essential. The diagnosis of TBM, like any other infectious disease, can be made by detecting the whole microorganism and/or its components or by host responses to the invading microorganism. Amongst the direct detection methods; microscopy of smears by differential or special staining is already in practice for *Mycobacterium tuberculosis (MTb)*, but is very poor in sensitivity since number of bacteria in the CSF is usually too low (<10^4/ml) and similar explanation is also true for poor sensitivity of *MTb*

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specific antigen detection.[2] However, cultivation and PCR amplification are the two methods whereby we can increase the number of copies and therefore sensitivity of detection improves. The conventional method of isolation of *MTb* in CSF is inadequate for early diagnosis as it takes 4-8 weeks apart from being very poor in sensitivity. However, the culture methods have been modified for early detection of the bacterial growth. The BACTEC method yields culture result as early as 7-10 days but has a limitation of disposal of radioactive wastes, incomplete automation and also requires expertise to perform the test.[3] BacT/ALERT 3D system, a colorimetric fully automated non-radiometric liquid culture system gives comparable results in relation with isolation of mycobacterium both in terms of sensitivity and time, in comparison to Bactec system.[4-7] Recently, nucleic acid amplification based assay (NAA), the method of increasing the copy number, has been found as a promising method for early diagnosis of tuberculosis because of its sensitivity, specificity as well as rapidity.[8-9] Several reports are available showing wide variation (65-100%) in ability of detection of *MTb* in TBM. On the basis of available reports, nested PCR assay has been found to be the most promising.[7-16] Real time PCR has been reported to be good, but it is less sensitive compared to nested PCR in detection of TBM.[16] Although, Takahashi et al.[16] have reported better detection of *MTb* by combining the real time (RT) PCR with nested PCR assay in CSF. However, later method is technically demanding. Available commercial serological tests provide inconsistent and imprecise results leading to highly variable sensitivity and specificity. Therefore, WHO has strongly recommended that these tests are not to be used for diagnosis of pulmonary and extra pulmonary TB. [17] The present study therefore was undertaken to evaluate the role of BacT/ALERT 3D culture system as well as nested PCR based detection of *MTb* for early diagnosis of TBM along with other conventional methods.

METHODOLOGY

The Present study was carried out in Departments of Medicine, Microbiology and Anesthesiology of the University Hospital of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India during the period of June 2009-May 2011. A total of 50 suspected cases of TBM and 20 healthy controls were included in the study. This study was approved by Institutional Ethics Committee and accordingly written informed consent was taken from each of participants or their attendants.

Case Selection: Patients with a compatible history and clinical/biochemical findings suggestive of TBM were included in the study. Supportive evidences were further collected by carrying out CSF – Adenosine deaminase activity (ADA), Monteux test, ESR, Chest X-ray (PA View), CT scan/ MRI of brain. The response to anti-tubercular therapy (ATT) also supported the clinical diagnosis of TBM. Patient with HIV infection or any immune-deficient state, autoimmune disease, viral encephalitis, diabetics, malignancy were excluded from the study.

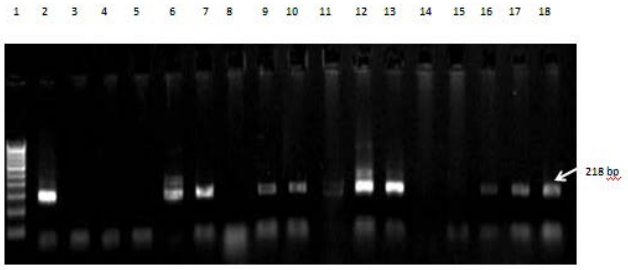
Controls: CSF was collected from patients undergoing spinal anaesthesia for surgical procedures for traumatic/non-infective condition as healthy controls.

Collection of cerebrospinal fluid: About 3.0-5.0 ml of CSF was obtained in a sterile vial from each of the patients and controls by performing lumbar puncture aseptically prior to start of ATT. Half of the volume (1.5-2.5ml) of collected CSF samples were subjected to the following: cytological and biochemical studies, AFB smear examination, BacT/ALERT MP culture immediately without centrifugation while other half was processed later for amplification by PCR for which CSF was preserved at 4 ^{0}C.

CSF Culture: Each CSF specimen was inoculated in the BacT/ALERT MP culture media (bioMerieux) by strictly following manufacturer's instructions. In brief: BacT/ALERT MP culture media [18] contains Middle rook 7H9 broth, pancreatic digests of casein, bovine serum albumin and catalase. BacT/ALERT MP media were brought to room temperature (RT) before inoculation. Disinfection of the top of each media septum was done with an alcohol pad (70% ethanol). Reconstituted antimicrobial agent supplement was added in the volume of 0.5 ml aseptically using a syringe and needle to each of the media. About 0.5ml each of the CSF sample was inoculated into the appropriately labeled MP media. The inoculated BacT/ALERT MP bottles were incubated in MB

cabinet. The positive bottles were further subjected to standard Lowenstein Jensen's medium and incubated for 12 weeks for further confirmation by morphological as well as molecular methods.

Extraction of DNA: To extract DNA from the CSF and BacT/ALERT positive culture media, the steps were followed as described by Sambrook et al.[19]



First lane- DNA ladder, Second lane- positive control, Third lane negative control, Lanes 3-32 suspected cases of tuberculous meningitis where lanes 6, 7, 9,, 10, 11, 12, 13, 16, 17 and 18 are showing amplicon of 218 bp size

Figure 1. Gel image showing 218 bp target amplicon from CSF after nested PCR targeting heat shock protein gene of Mycobacterium tuberculosis

Primers: Heat shock protein gene *(hsp65)* of *MTb* is known to be quite conserved and *in house* designed well tested primers hsp TB**F1** and hsp TBR1 were selected for amplification of a 494 bp nucleotide sequence for primary PCR; hspTB**F2** and hspTB**R2** were selected for amplification of a 218 bp nucleotide sequence for nested PCR.[20] The oligo sequences (5'-3') were as follows:

For Primary PCR:

hspTB*F1*: AAA AGC CGG ATG GCA ATT CG

hspTB*R1*: ATT ACC GGC TTG GAC CCC CTG

For Nested PCR:

hspTB*F2*: ATG CGG CGC ACA CCG AAG ACA AG

hspTB*R2*: TCA ACG CGC TGT CTA GCT TGT C

Buffers: *Taq polymerase* enzymes and customized primers were procured from SBS Genetech Co.,Ltd.,China.

Amplification of DNA: PCR reaction was carried out in 25 µl volume. Reaction mix contained 10 x reaction buffer (5 µl/sample), dNTPs (concentration 2.5 mmol each base), forward and reverse primers (concentration 10 pmol/ µl) and *Taq polymerase* enzyme (1U). Amplification was carried out on Biometra, Gottingen Thermo Cycler, Germany with a heated lid. The hot start method was employed by heating at 94^0C for 5 min initially. Thereafter, amplification was carried out for 35 cycles at 90^0C for 1min (denaturation), 65^0C for 1min (annealing) and 72^0C for 1min (extension). Extra extension was carried out at 72^0C for 7 min. The amplification products of primary PCR was again amplified with nested primers following same protocol. The final amplification products/bands were analyzed on 2 % agarose gel stained with ethidium bromide under UV light (figure 1). Positive (DNA extracted from *M. tuberculosis* growth) and negative (double distilled water) controls were run with each batch of samples analyzed. Documentation of gel was done by Multiimage™ Light Cabinet, Alpha Innotech Corporation, CA, USA.

Statistical methods: Mean, standard deviations were calculated for quantitative variables. Student Newman Keul Test, p-value and f-value have been used to test the significant difference between the mean of two groups. For qualitative and categorical variables, X^2 and Z-test have been applied to test the significant difference between two proportions. Sensitivity, specificity, positive and negative predictive value and accuracy were calculated at different cut off points of different variables in respect to PCR.

RESULTS

None of the CSF specimen was found positive for acid fast bacteria by Ziehl Neelsen staining.

Table 1. BacT /ALERT MP culture positivity in patients with TBM and healthy controls

	TBM Cases		Controls	
	No.	%	No.	%
BacT/ALERT Test(MP)				
Positive	38*	76	3**	15
Negative	12	24	17	85
CSF-PCR(nested)				
Positive	46#	92	1##	5
Negative	4	8	19	95

Fisher's Exact Test:
*versus ** $p < 0.0001$, # versus ## $p < 0.0001$, versus ## $p < 0.01$

BacT/ALERT MP culture of CSF showed that 76% of the specimens were positive in TBM while 15% of controls also yielded for the bacterial growth as indicated by the color change in the medium (table-1). However, detection rates between the two groups were significantly different (*P<0.0001*). The other interesting observation was that when DNA isolated from broth of BacT/ALERT MP culture was subjected for PCR amplification, 15 (39.5%) of them yielded the desired amplicon for *MTb.* Interestingly, when the liquid medium was inoculated to conventional Lowenstein Jensen's medium, 31.5% (12/38) were found positive for the growth of *M. tuberculosis* while rest of the BacT/ ALERT MP culture media yielded rapid growing contaminants mostly non acid fast bacteria. The PCR based amplification targeting heat shock protein gene (hsp65) of *MTb* by using nested protocol on CSF of TBM showed positivity in 92% (46/50) and in healthy controls positive amplification could be observed in 5% (table 1, figure 1). The detection rate in TBM was significantly higher than the controls (*P<0.0001*). Further, the detection rate by nested PCR based assay was significantly higher (*p<0.02*) as compared to BacT/ALERT MP Culture system.

DISCUSSION

Nucleic acid amplification assays (NAA) have been established as the best technique for rapid and accurate diagnosis of *MTb* cases in extra pulmonary infection including TBM. Several reports are available showing unsatisfactory sensitivity of detection (32% to 90%) of single amplification round which occurs mostly due to low bacterial count as well as presence of PCR inhibitors in biological samples.[7-16] Although, detection efficiency of real time PCR method is better than the gel electrophoresis, but the performance of this system is also marred by the PCR inhibitors and detection rate ranges between 17.5% to 93.8%.[16] The above two methods have usually been found to lack the desired accuracy in diagnosis of TBM.[21] However, recently introduced nested PCR assay has drastically improved the sensitivity and specificity of diagnosis TBM. The drawback of this system is that increased sensitivity is always associated with risk of contamination. There are several reports based on nested PCR targeting variety of structural and repetitive gene sequence of *MTb* often showing 100% sensitivity and specificity.[22] It has now been proved that nested PCR is 1000 times more sensitive than the conventional single round PCR assay. [21] Majority of the reports showing better results by nested PCR have targeted *MPT 64* gene sequences of *MTb*.[22] The *MPT 64* genes has been reported to be present exclusively in *MTb* and not even in BCG strain of Mycobacteria. Although, this makes it specific for *MTb,* it could not amplify the other members of *Mycobacterium* complex (M*. bovis, M. africanus* and *M. microti*) which are not rare in TBM. In the present study, we have targeted heat shock protein gene (hsp 65) which is much conserved and primers have been designed in such a way to cover all the four species of *Mycobacterium* complex. By using *in house* designed primers, we were able to detect, the *Mycobacterium* complex in 92% of CSF specimen of the suspected cases of TBM which is significantly better and faster than the culture method namely BacT/ALERT MP culture system which was found to be positive for the growth in 76% of the cases. Surprisingly, 5% of the CSF specimens collected from the healthy (Non tuberculous) individuals were also found positive for desired amplicon. Although, this detection of *MTb* may be explained on the basis of endemic proportion of tuberculosis in India, the fact that CSF is the second preferred site after lungs for the presence of *MTb* due to availability of high oxygen concentration and also because of the better detection sensitivity (1- 10 copy/ ml) of the present assay, which might be able to detect very low copy number of the bacterium. Thus, presence of *MTb* in CSF of healthy persons cannot be denied in endemic areas. However, it will be interesting to have follow-up of such cases and also further study involving large number of study subjects is essentially needed before drawing any conclusion. Another observation was that DNA isolated from broth of BacT/ALERT MP culture was subjected for PCR amplification, only 12/38 (31.5%) of them were found positive for the desired amplicon for *MTb.* One possibility for this observation may be that contaminant bacteria might have grown and this growth might be responsible for color change. Since the rest of the visibly positive bottles were observed with the contaminants growing, the efficacy of this medium becomes doubtful. Further there are reports that many of the saprophytic maycobacteria can also grow in this medium apart from normal commensal flora of the skin

inadvertently coming into the collection vials. Earlier studies also have shown high contamination rates of 17.1, 18.9, and 11.0% for BACTEC MGIT 960, ESP II, and Middlebrook agar, respectively when variety of specimens e.g. sputum, urine and stool were submitted for isolation to these automated culture systems.[23] Saprophytic mycobacteria. *M. gordonae* was the frequent isolate in these media.[23] This may lead to unnecessary administration of anti tubercular therapy to the patients if not confirmed by a definite confirmatory method. It may be likely that the inhibitors added to the BacT/ALERT MP culture may not be able to inhibit the contaminants. Presence of PCR inhibitors in this medium seems to be a remote possibility as those CSF specimens who were positive for *M. tuberculosis* complex specific amplification were also positive when PCR was performed from the liquid growth BacT/ALERT MP. Moreover, this observation could further be ratified by growing the *M. tuberculosis* by using conventional LJ medium in almost all the broth with the exception 3 culture broths only.

As all the suspected cases responded to anti tubercular treatment, clinical suspicion was considered as gold standard in the present study. The sensitivity, specificity, positive and negative predictive values of the presented nested PCR assay were 92%, 95%, 97.8% and 82.6% respectively. Although improved isolation is the ideal requirement, our PCR assay seems to be quite satisfactory in detection of *Mycobacterium* complex in CSF of the suspected cases of TBM. The sensitivity of detection by automated system seems to be disappointing and needs drastic improvement because of high rates contamination added with very high cost of establishment and consumable to run the service in TB diagnostics.

CONCLUSIONS

Nested PCR based detection of *M. tuberculosis* is definitely better than conventional culture isolation including BacT ALERT system. However, culture isolation has significant role for determination of drug resistance in *M. tuberculosis* the era of MDR and XDR tuberculosis as PCR based detection system is not available in most of the peripheral laboratories and also unable to detect drug resistance against all the anti tuberculous drugs in use. We have already sdeclared " No to TB serology". The better media for rapid growth are the need of the time. The available BacT ALERT system needs further improvement as observed in the present study since contaminates are also growing. Further, color change of the media should not be considered as an indicator of Mycobacterial growth in this culture system and the growth must be verified before giving the final report. However, study involving large number of patients and controls are warranted on the basis of the present study before drawing any definite conclusion.

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DELAY IN DIAGNOSIS OF TUBERCULOSIS AMONG UNDER TREATMENT PATIENTS IN RAJSHAHI CITY, BANGLADESH

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ABSTRACT

Introduction: Delay in diagnosis regarding Tuberculosis (TB) is common in developing country as well as in Bangladesh. Therefore, the aim of the study is to identify the factors which have effects on delay in diagnosis.

Methodology: A cross-sectional study was carried out at Rajshahi City, Bangladesh. A total of 384 TB patients were took part in the interview presented in the study areas for seeking treatment. The samples were selected using stratified random sampling with simple random sampling techniques. Descriptive statistics, chi-square test and multivariate logistic regression methods in SPSS were employed in this analysis.

Results: The results revealed that around 76.3% patients delay in diagnosis and among them 81.3% pulmonary patients were found delay. Patients' sex, educational status, area of living, and monthly family income were significantly associated with delay in the diagnosis of TB, as obtained through Chi-square test. Multivariate logistic regression method identified sex, educational status, monthly family income and first visit before diagnosis as risk factors of diagnostic delay.

Conclusion: Massive health education needs to be included in the existing TB control programs to enhance the accessibility of patients in health-care service without delay in diagnosis.

Key words: Tuberculosis, Diagnostic Delay, Socio-demographic Factors, Chi-square Test, Logistic Regression Analysis

INTRODUCTION

Tuberculosis (TB) is an ancient chronic communicable disease caused by the bacillus Mycobacterium TB (MTB) which spreads from person to person through air. It is the most common cause of infectious disease–related morbidity and mortality worldwide and also one of the top ten causes of global mortality. Reemergence and association with acquired immunodeficiency syndrome (AIDS) have made TB a global threat.[1]

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Currently, one third of world's population is infected with MTB resulting in annually approximately 1.8 million deaths worldwide.[2] TB remains a one of the major causes of morbidity and mortality in Bangladesh. Bangladesh is placed 6th among the 22 TB high burden countries (HBCs)[3] which focuses the susceptibility of TB in the country.[4] In 2010, the National TB Control Program (NTP), Bangladesh detected approximately 151,800 TB cases including 105,772 (70%) new smear-positive cases with case detection rate of 46.0%.[2] From 1993 onwards, the estimated case detection rate of the NTP has remained below the target of World Health organization (WHO) and diagnostic delay is one of the significant reasons behind this. The time interval from the appearance of the major symptoms of the disease until diagnosis is termed as diagnostic delay, which deteriorates the disease, boost the threat of morbidity and mortality

and enhance the risk of TB transmission in the community.[5,9] Early diagnosis of TB and rapid commencement of treatment is essential for an effective TB control program. An infected person who remains undiagnosed and untreated can infect ten to fifteen people every year.[10]

The magnitude and risk factors for diagnostic delays have been well documented in a number of studies.[1,11,12] Patient's decision on seeking healthcare depends on different factors.[13,14] In several studies, it has been documented that health seeking behavior, low access to health care facilities, poverty, rural residence, education, female sex, old age are in significant relation with delays in diagnosis.[1,5,15,16] Therefore, the authors have tried to find out the association between the various socio-demographic factors and delay in diagnosis among TB patients in Bangladesh context. In Bangladesh no recent study was available in this regard. Without identifying the appropriate reasons for diagnostic delay, it is difficult to minimize delay. Therefore, the main purpose of this study is to identify the determinants that affect patients' diagnostic delay for TB.

METHODOLOGY

Study Areas

A cross sectional study was carried out at Rajshahi City, Bangladesh. The study sample consists of 384 TB patients (284 pulmonary patients and 100 extra-pulmonary patients) at Rajshahi City, Bangladesh, between June 2011 and February 2012. Samples were selected using stratified random sampling with simple random sampling techniques. The Rajshahi City consists of four Thanas (sub-districts). One health care centre was chosen from each of four Thanas according to the good number of patients' flow. The selected health centers were Rajshahi Chest Disease Clinic (CDC) from Boalia Thana, Chest Disease Hospital (CDH) from Rajpara Thana, Tilottoma (Noudapara Branch) from Shah Mokhdum Thana and Population Service and Training Centre (PSTC) from Motihar Thana of Rajshahi city. All subjects gave their consent before the interview started.

Study Population Selection

The patients (>14 years old) were taking treatment during the study period were selected for this study. Patients in whom TB was suspected were sent to the laboratory for sputum microscopy and were registered in the TB laboratory. Patients diagnosed with smear positive TB were registered for treatment. Pulmonary TB was diagnosed according to the Guidelines NTP Bangladesh.[17] The diagnosis of extra-pulmonary TB was based on a combination of clinical, radiological, and histopathological findings. In these selected study sites, TB is diagnosed and treated without charge and the result of the diagnosis was confirmed in the same day.

Survey Instrument, Data Management and Analysis

The patients were face-to-face interviewed by using a semi-structured interview schedule containing pre-coded and open-ended questions. Required corrections were made following an evaluation of the pilot survey and a final version of the questionnaire was completed. Two trained interviewers and a medical doctor conducted the interviews after obtaining informed consent of the respondent. Patients were informed clearly about the purpose of the study. For each patient, the following information was collected: sex, age, educational status, family income, area of living, type of house, type of patient, first visit before diagnosis and duration of suffering. In this study, diagnostic delay was considered when the time interval between the onset of symptoms and diagnosis was more than three weeks.

Univariate analysis was done to find out the distributions of different items related to diagnostic delay. Cross study comparisons were carried out between diagnostic delay and socio- demographic characteristics. The Pearson's Chi-squared (χ^2) test for higher contingency tables was used to determine associations between dependent and independent variables. Binary logistic regression analysis was performed to identify the socio-demographic determinants that affect patients' diagnostic delay. The independent variables considered in this study were age, sex, educational status, monthly family income, area of living, type of house, type

of patients, and first visit before diagnosis. The dependent variable has been classified in the binary logistic regression model as follows:

$$Y=\begin{cases}1, & if\ the\ patients\ delay\ in\ diagnosis\\ 0, & otherwise\end{cases}$$

All statistical analyses were performed using Statistical Package for Social Science (SPSS, version 16).

RESULTS

Socio-economic and Demographic Characteristics of Patients

Table 1 represents socio-economic and demographic characteristics of TB patients. Among the selected 384 TB patients, more than half (58.6%) were males, most patients (67.7%) were found between ages 20-50 years. About half of the Patients (52.6%) had lower level of education (completed 0 to 5 years of education). The higher percentage of TB patients were found among low income group (monthly family income <8000 TK) (46.4 %), town dwellers (61.5%), whose houseswere brick built (57.0%). In case of patients type, more than two third were pulmonary patients (74.0%) and suffer from $\leq$6 months (69.3%). Also higher percentage of patients (41.9%) went to public hospital for seeking healthcare before diagnosis of TB disease. Most of these patients (76.3%) reported diagnostic delay.

Table 1. Background Characteristics of TB Patients

Factors	Frequency	Percent
Sex - Male	225	58.6%
Female	159	41.4%
Age - <20 years	42	10.9%
20-35 years	138	35.9%
36-50 years	122	31.8%
>50 years	82	21.4%
Educational status		
0-5 years of schooling	202	52.6%
6-12 years of schooling	128	33.3%
>12 years of schooling	54	14.1%
Monthly family income (BDT)		
<8000	178	46.4%
8000-15000	88	22.9%
>15000	118	30.7%
Area of living - Village	148	38.5%
Town	236	61.5%
Type of house - Clay made	165	43.0%
Brick built	219	57.0%
Type of patients		
Extra-pulmonary	100	26.0%
Pulmonary	284	74.0%
First visit before diagnosis		
Pharmacy	79	20.6%
Public hospital	161	41.9%
GO/NGO clinic	144	37.5%
Duration of suffering		
$\leq$ 6 months	266	69.3%
> 6 months	118	30.7%
Delay in diagnosis - No	91	23.7%
Yes	293	76.3%
Total	**384**	**100**

Associations between Diagnostic Delay among TB Patients and Various Socio-demographic Factors

Table 2 represents the associations between diagnostic delay among TB patients and different socio-demographic factors along with the significant values. The associations were obtained through Chi-square test between diagnostic delay and the socio-demographic factors of the patients. All the variables except age were significantly associated with diagnostic delay among the TB patients. The study results revealed that, 80% of all male patients reported delayed diagnosis (>3 weeks), which was comparatively higher than that of females (71.1%). The results also noticed that, diagnostic delays increased with the increase of ages; but it is decreased with the increase of the levels of education. The 69% of patients aged below 20 years reported diagnostic delay, which has sequentially increased to 80.5% for patients aged over 50 years. Again, the higher percentage of diagnostic delay (84.7%) were found among respondents who had completed 0 to 5 years of schooling, but the percentage was lower among respondent who had completed 6 to 12 years of schooling (68.8%) and above 12 years of schooling (63.0%).

Delay in the diagnosis of TB disease was more common among the patients from village area compared to the patients form urban area (70.3%). The percentage of patients that experienced diagnostic delay was significantly higher among pulmonary patients (81.3%) and who lived in clay made house (84.8%) compared to extra-pulmonary patients (62.0%) and those lived in brick built

Table 2. Associations between Diagnostic Delay among TB Patients and Various Socio-demographic Factors

Factors	Diagnostic delay		P values
	Not delay (≤ 3 weeks)	Delay (> 3 weeks)	
Sex			
Male	45 (20.0%)	180 (80.0%)	0.043
Female	46 (28.9%)	113 (71.1%)	
Age			
<20 years	13 (31.0%)	29 (69.0%)	0.570
20-35 years	33 (23.9%)	105 (76.1%)	
36-50 years	29 (23.8%)	93 (76.2%)	
>50 years	16 (19.5%)	66 (80.5%)	
Educational status			0.000
0-5 years of schooling	31 (15.3%)	171 (84.7%)	
6-12 years of schooling	40 (31.2%)	88 (68.8%)	
>12 years of schooling	20 (37.0%)	34 (63.0%)	
Monthly family income (BDT)			0.000
<8000	22 (12.4%)	156 (87.6%)	
8000-15000	26 (29.5%)	62 (70.5%)	
>15000	43 (36.4%)	75 (63.6%)	
Area of living			
Village	21 (14.2%)	127 (85.8%)	0.001
Town	70 (29.7%)	166 (70.3%)	
Type of house			
Clay made	25 (15.2%)	140 (84.8%)	0.001
Brick built	66 (30.1%)	153 (69.9%)	
Type of patients			
Extra-pulmonary	38 (38.0%)	62 (62.0%)	0.000
Pulmonary	53 (18.7%)	231 (81.3%)	
First visit before diagnosis			0.000
Pharmacy	8 (10.1%)	71 (89.9%)	
Public hospital	25 (15.5%)	136 (84.5%)	
GO/NGO clinic	58 (40.3%)	86 (59.7%)	
Duration of suffering			0.001
≤ 6 months	76 (28.6%)	190 (71.4%)	
>6 months	15 (12.7%)	103 (87.3%)	
Total	**91(23.7%)**	**293 (76.3%)**	

house (69.9%) respectively. but diagnostic delay were found nearly same for both patients who went to pharmacy (89.9%) and public hospital (84.5%) for seeking healthcare before the diagnosis of TB disease. Delay in the diagnosis was more prevalent among the patients who suffered from TB more than 6 months (87.3%).

Risk Factors for Delay in Diagnosis of TB among Patients

Table 3 represents the estimate of relative risks (Odds ratio [OR]) that were calculated for each of the categorical variables, p-value, and 95% confidence interval (CI) for OR. In this study, the logistic regression model has beenfitted by respondent's age, sex, educational status, monthly family income, area of living, type of house, type of patients, and first visited healthcare center before diagnosis. The result of logistic regression analysis revealed that, sex had significant effect on diagnostic delay. Female patients had about 42% [OR = 0.582, 95% CI = 0.329-1.028] lower probability of delayed in diagnosis of TB than male patients. The study result indicated that, educational status had negative and significant effect on the diagnostic delay. The study result showed that education had somewhat significant impact on delay in diagnosis since the p value (p=0.067) was close to the significant level for the patients who completed 6-12 years of schooling. The patients who had completed 6-12 years of schooling had about 52% (OR=0.481, 95% CI = 0.220-1.053) lower risk of delayed in diagnosis than those of less educated (completed 0 to 5 years of schooling) patients. Again, the patients whose monthly income was 8000-15000 TK had 64% less risk (OR= 0.355, 95% CI= 0.155-0.811) and whose monthly family income was >15000 TK had 57% less risk (OR= 0.433, 95% CI= 0.163-1.152) of having diagnostic delay compared to those having monthly family income below 8000 TK. The result also indicated that, the patients who went to GO/NGO clinic for seeking healthcare before diagnosis of TB had about 80% less probability [OR= 0.206, 95% CI = 0.087-0.489] of having diagnostic delay compared to those went to pharmacy for seeking health care.

Table 3. Risk Factors of Delay in Diagnosis among TB Patients

Explanatory variables	B	S.E.	WS	P-values	OR (95% CI)
Sex					
Male (RC)					1.00
Female	-0.542	0.290	3.483	0.062	0.582 (0.329-1.028)
Age					
<20 years (RC)					1.00
20-35 years	-0.165	0.439	0.142	0.706	0.848 (0.359-2.003)
36-50 years	-0.526	0.465	1.280	0.258	0.591 (0.238-1.470)
>50 years	-0.287	0.506	0.323	0.570	0.750 (0.278-2.021)
Educational status					
0-5 years of schooling (RC)					1.00
6-12 years of schooling	-0.732	0.400	3.350	0.067	0.481 (0.220-1.053)
>12 years of schooling	-0.751	0.496	2.293	0.130	0.472 (0.178-1.248)
Monthly Family income (BDT)					
<8000 (RC)					1.00
8000-15000	-1.036	0.422	6.031	0.014	0.355 (0.155-0.811)
>15000	-0.837	0.499	2.811	0.094	0.433 (0.163-1.152)
Area of living					
Village (RC)					1.00
Town	-0.032	0.415	0.006	0.938	0.968 (0.430-2.182)
Type of house					
Clay made (RC)					1.00
Brick built	0.504	0.474	1.132	0.287	1.656 (0.654-4.193)
Type of patients					1.00
Extra-pulmonary (RC)					
Pulmonary	0.293	0.316	0.861	0.354	1.340 (0.722-2.489)
First visit before diagnosis					
Pharmacy (RC)					1.00
Public hospital	-0.487	0.444	1.204	0.272	0.614 (0.257-1.467)
GO/NGO clinic	-1.580	0.441	12.854	0.000	0.206 (0.087-0.489)
Constant	3.027	0.709	18.216	0.000	20.630

DISCUSSION

The study showed that TB patients at Rajshahi City, Bangladesh suffer from substantial delays until a diagnosis is made. Respondent's sex, educational status, family income, area of living, types of house, types of patient, first visited healthcare center before diagnosis and duration of suffering are significantly associated with the diagnostic delay of patients. In accordance with other studies[1,5,18,19] significant association were found among socio-economic factors and patients' diagnostic delay. Studies conducted in Tanzania and Botswana showed that patients from village areas, patients with low education level, site of first visit, lack of TB information and female sex were associated with TB delay.[20] A study in South India found longer diagnostic delay among men.[21] Another study in Nepal summarized that male patients experienced higher diagnostic delay[22] but several studies in developing countries reported longer diagnostic delay among women.[19,23] In our study, male patients experienced remarkable delay in diagnosis and constituted the higher percentage as compare to female patients. But the result does not match with other studies which revealed that propensity to make delay was higher among females.[5,16,20,24]

Several studies has highlighted that illiteracy was significantly associated with diagnostic delay. [25-27] Our study revealed that education is one of the most significant factors that closely associated with delay in diagnosis among patients. Lower educated patients (0-5 years of schooling) constituted the higher number and low level of education has somewhat significant impact on patient's diagnostic delay as well. Diagnostic delay was found low among the higher educated (>12 years of schooling) patients. A qualitative approach at rural area of Tanzania did not found any patients being higher educated.[28] The result of present study was consistent with some other studies [5,7,29,30] as well. Economic barrier frequently causes obstruction for seeking health care facilities. Patients especially from village area, often go far away for seeking health care due to poor diagnostic facility which is sometimes cost ineffective for them that resultant delay in diagnosis.[18] This study resulted that the patients correspond to comparatively low income group at Rajshahi, found delay in diagnosis of TB. Some previous studies found the same result. [13,18,22] Among the demographic factors, area of living has been identified significant for the diagnostic delay in this study. The patients' delay in diagnosis varied with the patients' area of residence. In our study, the percentage was higher for the patients of

rural setting as compared to urban setting. Several studies highlighted that higher percentage of TB patients reported delay corresponded to rural area [15,31] which was similar to the findings of present study. The delay in diagnosis in rural areas is higher due to low level of knowledge and awareness of the disease, lack of information about availability of free treatment, income and communication constraints of villagers. Delayed diagnosis was documented for both extra-pulmonary and pulmonary TB patientsand it was more prevalent among pulmonary TB patients in the present study. Many authors have found higher diagnostic delay among pulmonary patients.[1,5,30] But, in accordance with some other studies[10,11,16] extra-pulmonary TB patients were significantly more likely to have experienced diagnostic delay. However, it is not surprising because, patients with extra-pulmonary TB may have diffuse or non-specific symptoms and are likely to experience longer delays than patients with pulmonary TB in both low and high-endemic settings.

Different studies identified poor access to the NTP as one of the main factors in delayed diagnosis.[5,15,23-32] In this study, the higher percentage of patients went to public hospital for seeking health care, but diagnostic delay were higher among those went to pharmacy at first for seeking health care before the diagnosis. A study in Ethiopia, patients who first visited a qualified medical provider experienced delay before initiation of diagnosis and treatment.[32] After the onset of symptoms especially the rural people of Bangladesh go to pharmacy or traditional practitioner due to lack of knowledge, financial and communication problems, and sometimes due to negligence of the disease as well as due to poor coverage of NTP. Sometimes these cause delay in diagnosis. A bunches of studies have revealed that selection of a traditional practitioner for the first visit is associated with a prolonged delay in diagnosis.[19,23,24,33] In this study, two-third of the patients recorded living with TB within 6 months and the percentage that experienced diagnostic delay was higher for the patients who suffered TB for more than 6 months which indicates the vulnerability of diagnostic delay. Several studies were conducted on the new TB patients and found significant delay in diagnosis[1,5,8,32] and no recent study was found on old TB patients for delay in diagnosis. For the patients, delay in diagnosis and the late start of TB treatment can result in increased severity and mortality.[11] In our study, multivariate analysis has noticed that lack of education, poor income, pulmonary TB are the risk factors for diagnostic delay which is supported by the other studies. [5,16,18]

Limitation of the Study

There were several limitations to our study. Firstly, TB patients aged below 14 years were not included in this study. Because all the patients below 14 years of age were found children during the study period and the information were collected for the study was not enough to study the child patient's delay in diagnosis. Some other variables are essential along with the present information to serve the purpose. Further research should be conducted on the concern issue precisely. Secondly, only four healthcare centers at Rajshahi city were selected for this study. The sample size was relatively small but they were adequate for evaluating requisite purpose.

DISCUSSION

This study demonstrated that some socio-demographic factors have significant effects on delay in diagnosis of patients. Patients' sex, educational status, family income, area of living, type of house, type of patients, first visit before diagnosis and duration of suffering are significantly associated with diagnostic delay. Significant delay was found among the patients of low educated and lower income group. The patients who first visited public and private hospitals found delay in diagnosis. The multivariate analysis illustrated that patient who first visits pharmacy experienced delay in diagnosis. Therefore, it is important to facilitate prompt utilization of the health services by raising public awareness about the disease. Also, the existing TB control programs need to be modified and massive health education system need to be included in the existing TB control programs.

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LIFESTYLE PATTERN AMONG THE PEOPLE LIVING WITH AIDS IN EASTERN NEPAL

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ABSTRACT

Introduction: In world more than 40 million people are living with HIV/AIDS, 2.3 million are under 15 yrs and 14000 new infections occur daily. The objectives of this study was to assess the lifestyle pattern among the people living with AIDS (PLWA) receiving anti-retroviral therapy at B. P. Koirala Institute of Health Sciences and find out the association between lifestyle pattern and the selected demographic variables.

Methodology: A descriptive cross-sectional research design was used to assess lifestyle pattern of the PLWA receiving anti-retroviral therapy at the ART clinic of Tropical ward at B.P Koirala Institute of Health Sciences (BPKIHS). The PLWA receiving ART at BPKIHS for more than or equal to three months at the ART clinic who met the selection criteria constituted the sample of the study. Using purposive sampling technique total 113 PLWA were selected. The patients who themselves were physically present during the data collection period were included in the study, after their consent for the purpose.

Results: It was found that most of the PLWA were of age less than 40 years, male (61.1%), residing in urban area (74.3%), married (75.2%) and Hindu (74.3%). It was found that 83.2% PLWA never do exercise, whereas 8.8% perform regularly. Most of the (91.2%) people living with AIDS reported feeling pressure and stress in daily life. About 57% PLWA only sleep less than 6 hours per day. About half (52.2%) of the PLWA never take food outside home, where as 34.5% take food outside regularly. The habit of using tobacco is present among 28.3% people living with ADIS, where the past history of using was 39.8%. Similarly only 0.9% PLWA had habit of taking alcohol at present and 63.7% in past; and 28.3% had habit of taking substances in past. The association calculated between selected demographic variables (age, sex, marital status, residence and religion) with lifestyle pattern (exercise, tobacco chewing, alcohol consumption, and food habit) found significant association between marital status and exercise ($p < 0.001$), Tobacco consumption and age ($p < 0.001$) only.

Conclusion: The positive life style practices among the PLWA found high at present in comparison to past especially in relation to Tobacco, Alcohol and Substance use. Individual counseling and support is vital for improving the condition.

Key words: Life-style, People Living with AIDS, Counseling, ART

INTRODUCTION

HIV/AIDS is a global epidemic which first emerged in 1981 in the USA. Since then, the epidemic has claimed lives of nearly 30 million people worldwide, the worst conditions being in the Sub- Saharan countries.[1]

South Africa is reported to have the largest population living with the disease. In terms of prevalence, countries such as Afghanistan, Saudi Arabia, and Cape Verde are reported to have the lowest prevalence of the disease among reported nations, at less than 0.1% of their population.[2] According to UNAIDS/WHO, 47% of the established 14.2 million people eligible for the treatment in low

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and middle income countries were accessing ART (Anti Retro Viral) therapy in 2010.[3]

The AIDS picture in South East Asia is dominated by the epidemic in India. Migrants in particular are vulnerable and 67% of the infected in Bangladesh and 41% in Nepal are migrants returning from India.[4]

In a study done by the Department of Foods, Nutrition and Dietetics, Kenyatta University, among the HIV/AIDS infected two districts of South Africa , it was found out that majority of PLWHA consume foods that are low in nutrients to build up the immune system and help maintain adequate weight, and there is little variety in the foods they consume.[5] In fact, the linkages between HIV/AIDS and food security are bi-directional: HIV/AIDS is a determining factor of food insecurity as well as a consequence of food and nutrition insecurity.[6]

METHODOLOGY

A descriptive cross sectional research design was used to assess lifestyle pattern of the people living with AIDS receiving anti-retroviral therapy at the ART clinic of Tropical ward at BPKIHS. The PLWA receiving ART at BPKIHS for more than or equal to three months at the ART clinic who met the selection criteria constituted the sample of the study. Total 113 PLWA were selected using purposive sampling technique. The patients who themselves were physically present during the data collection period were only included in the study, with their consent for the purpose.

RESULTS

It was found that most of the PLWA were of age less than 40 years, male (61.1%), residing in urban area (74.3%), married (75.2%) and Hindu (74.3%). It was found that 83.2% PLWA never perform the exercise, whereas 8.8% perform regularly. Majority (91.2%) of the PLWA expressed the feeling of pressure and stress in daily life. About 57% PLWA only sleep less than 6 hours per day. About half (52.2%) of the PLWA never take food outside home whereas 34.5% take regularly. PLWA residing in eastern Nepal receiving ART at BPKIHS had habit of taking tobacco more in past (39.8%) in comparison to present (28.3%). Similarly only 0.9% PLWA had habit of taking alcohol at present and 63.7% in past; and 28.3% had habit of taking substances in past. The association calculated between selected demographic variables (age, sex, marital status, residence and religion) with lifestyle pattern (exercise, tobacco chewing, alcohol consumption, and food habit) found significant association between marital status and exercise ($p < 0.001$), and Tobacco consumption and age ($p < 0.001$) only. The details of the results are depicted in the table 1 to 4.

Table 1. Socio-demographic Characteristics of the People Living with AIDS (n=113)

SN	Socio-demographic Characteristics	Responses	
		Number	%
1	**Age group (in years)**		
	≤ 25	15	04.4
	26- 30	19	16.8
	31- 35	35	31.0
	36- 40	30	26.5
	41- 45	12	10.6
	≥ 46	12	10.6
	Mean = 36.3 SD= 8.268 Range= 19-7		
2	**sex**		
	Male	69	61.1
	Female	44	38.9
3	**Residence**		
	a. Rural	29	25.7
	b. Urban	84	74.3
4	**Marital Status**		
	a. Unmarried	85	75.2
	b. Married	15	13.3
	c. Divorced	12	10.6
	d. Widow	01	0.9
5	**Religion**		
	a. Hindu	84	74.3
	b. Buddhist	17	15.0
	c. Muslim	1	0.9
	d. Christian	5	4.4
	e. Kirata	6	5.3
7	**Family Income / Month (in Rupees)**		
	4000- 14000	26	23.01
	15000- 25000	26	23.01
	26000- 36000	21	18.58
	37000- 47000	23	20.35
	≥48000	17	15.04
	Mean = 29,982.30 SD= 18, 968.010 Range = 4000- 80,000		

Table 2. Distribution MI of the Respondents according to HIV Status, Assessed Dietary Pattern & B (n= 113)

SN	Characters	Categories (in months)	ART consumption Frequency (%)
1	Duration of ART consumption	3 – 12	30 (26.55)
		13 – 36	42 (37.17)
		37- 60	35 (30.97)
		> 60	6 (5.31)
Mean = 33.13, SD = 23.735, Range = 3-142			
2	CD4 count At the Start of Therapy	≤ 50	10 (8.85)
		51 – 250	79 (69.9)
		251- 500	23 (20.35)
		≥ 501	1 (0.9)
Mean = 184.24, SD = 101.692, Range = 2- 524			
3	Diet Habit	Non-Vegetarian	112 (99.1)
		Vegetarian	1 (0.9)
4	Frequency of Eating/ day	3times	6 (5.31)
		4 times	17 (15.04)
		5 times	74 (65.49)
		≥6times	16 (14.16)
5	Composition of Major Meals is mostly	Carbohydrates	111 (98.23)
		Proteins	2 (1.77)
6	Nutritional Supplement Consumption (NCASC)	Positive response	29 (25.66)
7	**Body Mass Index (BMI)**	**Frequency**	**Percentage**
	< 18.5 (malnourished)	28	24.79
	18.5 – 24.9 (normal)	73	64.60
	25- 29.9 (overweight)	12	10.62
	≥30	0	0
Mean = 20.85, SD = 3.13, Range = 14.17- 29.56			

Table 3. Lifestyle Pattern, Tobacco Chewing, Smoking Habit, Alcohol & Drug Consumption Habit among the PLWA (n=113)

SN	Lifestyle Pattern	No.	%
1	Exercise habit:		
	a. Daily	10	8.8
	b. Alternate day	2	1.6
	c. 1-2/week	7	6.2
	d. Never	94	83.2
2	Feeling under pressure and stress:		
	a. Often	103	91.2
	b. Occasionally	2	1.8
	c. Never	8	7.1
3	Hours of sleep per day:		
	a. < 6 hrs	48	42.5
	b. 6-8 hrs	64	56.6
	c. >8 hrs	1	0.9
4	Taking food outside the home:		
	a. Daily	39	34.5
	b. Alternate day	11	9.7
	c. 1-2/week	4	3.5
	d. Never	59	52.2
5	Smoking/Tobacco Chewing habits:		
	Present history:		
	a. Yes	32	28.3
	b. No	81	71.7
	Past history:		
	a. Yes	45	39.8
	b. No	68	60.0
	Present consuming habits of different:		
	a. Chewing tobacco	19	11.8
	b. Cigarette smoking	13	11.5
	c. Betal chewing	0	0
	d. Hooka/Chilim	0	0
	e. Bidi smoking	0	0
6	Present habit of Alcohol consumption		
	a. Yes	1	0.9
	b. No	112	99.1
7	Past habit of alcohol consumption		
	a. Yes	41	36.3
	b. No	72	63.7
8	Current habit of consumption of different types of alcohol		
	a. Beer	0	0
	b. Wine	0	0
	c. Rakshi/Jand	1	0.9
	d. Tongba	0	0
	e. Whiskey/Rum	0	0
9	Drug abuse habit:		
	a. Habit in present	0	0
	b. Habit in past	32	28.3

Table 4. Association between Selected Demographic Variables and lifestyle Patterns (n = 113)

	Characteristics	Categories	Lifestyle Pattern														
			Exercise			Feeling Pressure			Sleep			Tobacco Consumption			Alcohol Consumption		
			Others	Daily	P-value	Occasionally / never	Often	P-value	Others	6-8 hrs	P-value	No	yes	P-value	No	yes	P-value
Demographic Characteristics	Age	>41	21	71	0.983	0	8	0.128	1	48	<0.001	14	67	0.102	24	88	0.602
		<41	3	16		24	81		23	41		10	22		0	1	
	sex	Female	36	56	0.471	3	5	0.931	16	33	0.231	30	51	0.510	43	69	0.208
		Male	6	13		41	64		28	36		14	18		1	0	
	Residence	Urban	72	2	0.072	6	2	0.964	39	10	0.263	61	20	0.707	83	29	0.555
		Rural	12	7		78	27		45	19		23	9		1	0	
	Marital Status	UM/ D/W	11	3	<0.001	0	8	0.092	13	36	0.706	21	60	0.653	28	84	0.569
		Married	17	2		28	77		15	49		7	25		0	1	
	Religion	B/C/M/K	21	73	0.072	0	8	0.085	13	36	0.854	22	59	0.562	28	84	0.087
		Hindu	8	11		29	76		16	48		7	25		1	0	

DISCUSSION

It was found that most of the (31%) PLWA were of age group of 31-35 years, male (61.1%), residing in urban area (74.3%), married (75.2%) and Hindu (74.3%). Study conducted by Sharma[7] reported that out of 150 patients 66.7% were male, 33.3% were females. Most of them (41.3%) were illiterate, married (49.3%), and farmer (35.3%). The age group 21-30 years was predominant followed by 31-40 years (42%); which is similar to this study. Similar findings were also reported by Budhachandra and Dhungana.[8,9]

It was found that 83.2% PLWA never perform the exercise, whereas 8.8% perform regularly. About 57% PLWA only sleep less than 6 hours per day. About half (52.2%) of the PLWA never take food outside home whereas 34.5% take regularly.

PLWA residing in eastern Nepal receiving ART at BPKIHS had habit of taking tobacco more in past (39.8%) in comparison of present (28.3%). Similarly only 0.9% PLWA had habit of taking alcohol at present and 63.7% in past; and 28.3% had habit of taking substances in past. Similar study conducted by Dhungana[9] reported, 41% PLWHA were smoker, 34% Alcoholics and 54% had weight loss, which is similar to this study.

The association calculated between selected demographic variables (age, sex, marital status, residence and religion) with lifestyle pattern (exercise, tobacco chewing, alcohol consumption, and food habit) only found significant association between marital status and exercise ($p < 0.001$), and Tobacco consumption and age ($p < 0.001$).

CONCLUSION

The positive life style practices among the PLWA found higher at present in compression to before illness. Individual counselling and support is vital for improving the condition.

Limitations: Due to the proxy visits by the relatives instead of the patient, the sample collection was difficult as anthropometric measurements could not be taken. There was difficulty in obtaining data about the dietary patterns and lifestyle. The setting of the clinic could not ensure proper privacy. Patients who came in a hurry couldn't be assessed in detailed related to their short stay at clinic.

Recommendations: The supplementation of the nutrition provided by WHO and distributed by the ART clinic can be improved and the target group can be encouraged to consume it. Similar study can be conducted at other ART centers and results can be compared. This study can be done on a larger sample and the association with diet patterns and lifestyle can be observed, which also affect the nutritional status in one way or the other. The BMI at the start of the therapy can be assessed and used to compare the improvement after the initiation of ART.

Implications of the study: This study gives an overview of the nutritional status of the PLWHA receiving ART at BPKIHS. For patients at the risk of being malnourished, ART initiation can be an important step in attempt to increase their nutritional status.

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Case study

LARGE PRIMARY TUBERCULOUS PSOAS ABSCESS IN AN HIV PATIENT – A CASE REPORT

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ABSTRACT

Tuberculosis (TB) remain as one of the leading opportunistic infection in patients with Human immunodefficiency virus (HIV) infection in developing countries. Here we report a case of huge primary psoas abscess of tubercular origin in an HIV patient who presented with hip pain and limping. Psoas sign was evident on examination and CT scan of abdomen revealed a large iliopsoas abscess extending from abdomen to upper part of thigh. Patient underwent percutaneous drainage of abscess and improved with anti-tuberculous drugs while continuing anti-retroviral therapy.

Key words: HIV, AIDS-Related Opportunistic Infections, Psoas Abscess, Tuberculosis

INTRODUCTION

Human immunodeficiency virus (HIV) and Tuberculosis (TB) are the first and second most cause of death due to an infectious disease and in 2012, 0.3 million HIV-associated TB deaths were reported worldwide.[1] HIV and TB accelerate their course each other increasing the mortality and morbidity rates. Unusual presentations of TB due to altered immune response pose diagnostic challenge in HIV. Clinical manifestations due to the underlying TB depends on the CD4+ count- patients with higher counts may present with cavitatory upper lobe lung lesions or caseating lymph nodes and those with lower counts develop more disseminated disease like military TB. Here is an account of a patient with HIV on highly active anti-retroviral therapy (HAART) and developed relapse of TB after two years in the form of huge iliopsoas abscess. The case is presented considering the sheer size of the abscess, recovery with treatment and also to remind the possibility of psoas abscess in HIV patients with hip pain.

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Case report

32 year old male, HIV-1 positive, presented with history of left hip pain and limp on walking for 3 weeks. On examination, he was afebrile and vital signs were normal. There was no pallor, lymphadenopathy or oral candidiasis. Chest was clear to auscultation and abdomen was soft without any palpable mass. His left limb was kept in a partial hip flexion and was painful on forced extension (Psoas sign, figure 1). Patient was on Zidovudine 300 mg, Lamivudine 150 mg, and Nevirapine 200 mg; all tablets twice daily for the last 2 years with improving CD4+ count. He was treated for TB involving the intra-abdominal lymph nodes two years back and at that time CD4+ count was 80/mm^3(500-1300/mm^3). This time CD4+ count was 270/mm3 (Viral load was not done).CT scan of abdomen with contrast revealed a large lobulated thin walled fluid collection in the left iliopsoas compartment from the level of disc between first and second lumbar vertebrae to about six cm below the left lesser trochanter (figure 2 and 3). Nearby vertebrae and disc spaces were intact. Chest X ray was normal. Abscess was sampled percutaneously

through a needle and yielded yellowish caseating material which stained negative for acid fast or gram positive bacilli. In view of caseating nature of the aspirated material, absence of any other bacilli in gram stain and radiological features, a diagnosis of primary tubercular psoas abscess was made. The abscess was approached.

percutaneously through a small incision posteriorly just above the left iliac crest and about 500 ml of pus was drained. He was put on WHO Category II anti-tuberculous regimen. HAART was continued; Nevirapine was replaced with Efavirenz 600 mg once daily. Patient improved over a period of 4 months and at one year he is very well ambulant with a small limp.

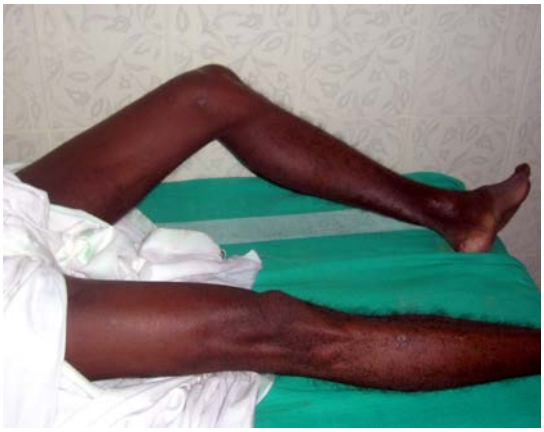

Figure 1. Left hip kept in a semi flexed position due to painful spasm of psoas muscle

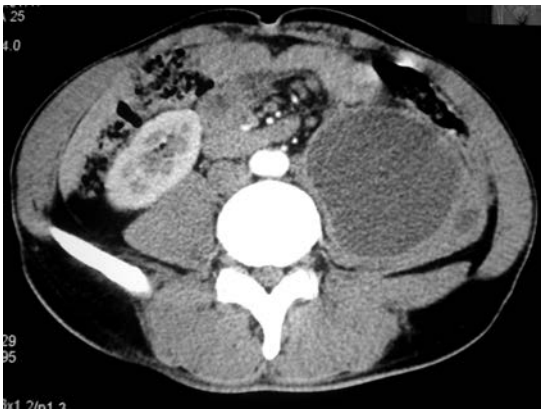

Figure 2. CT scan showing the huge abscess of the left iliopsoas muscle

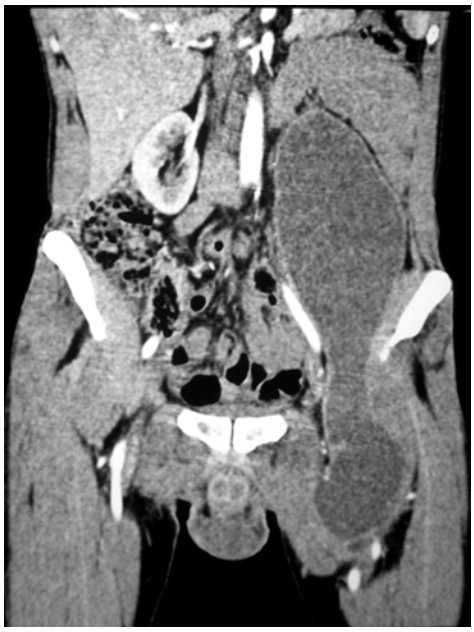

Figure 3. CT scan showing the huge abscess of the left iliopsoas muscle

DISCUSSION

Psoas and iliacus muscle-together called as iliopsoas- are located in the iliopsoas compartment and originate form the lateral aspects of 12th thoracic to 5th lumbar vertebrae, to be inserted to the lesser trochanter of femur. Infections can enter into this muscle form it's neighboring structures like iliac lymph nodes, sigmoid colon and abdominal aorta.

Psoas abscess in immune competent patients is either primary or secondary due to peri-nepheric abscess, lumbar discitis or infected abdominal aortic aneurysm.[2] *Staphylococcus aureus* remain as the most common aetiological agent and others are *Escherichia coli*, *Klebseilla pneumonia*, *Enterobacter* and *Mycobacterium tuberculosis*.[3]

Most of the time, the tuberculous psoas abscess is secondary; source of infection being osteomyelitis of the spine.[3] The primary psoas abscess is rare especially in HIV patients with only few case reports.[4]

The Psoas abscess may present with pain involving back, hip or lower abdomen. Patient can develop a limp with pain radiating to back of thigh. Fever will be present in majority and psoas abscess remains as one of the causes for fever of unknown origin- in a case series, median time between symptom onset and diagnosis was >42 days in one third of the patients.[5]

Diagnosis is established with MRI or a contrast enhanced CT scan of abdomen as ultrasound is a less sensitive method. Sampling of the material can be done with needle aspiration. Though classical treatment remain as open surgical drainage[6], laproscopic[7] and percutaneous drainage are also described with successful outcome.[8] Most of them recover but sequel can occur due to osteomyelitis of adjacent bones and scarring or weakness of muscle.[9]

Patient in our case had abdominal lymph node TB during the period of lower CD4+ count (before initiating HAART). After two years of HAART, TB relapse took the form of caseating mass, most likely due to higher CD4+ count (Immune reconstitution). The case is presented due to several unique features- patient presented with hip pain rather than fever, it was a primary abscess and had a good outcome with open drainage and anti-tuberculous drugs.

CONCLUSIONS

With the advent of HIV, the psoas abscess due to TB is expected to increase in developing countries. The diagnosis, especially in earlier stages can be challenging as blunted immunological response may not produce noticeable signs of infection in HIV patients. Primary tuberculous psoas abscess in HIV patients remain rare and there there are no definite guidelines regarding the treatment as there are only a very few case reports. In patients with HIV, the possibility of this entity should be kept in mind in cases of persistent hip pain.

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Case study

TUBERCULOSIS OF ORAL MUCOSA - A CASE REPORT

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ABSTRACT

Tuberculosis (TB) is a chronic granulomatous disease and a leading cause of death in developing countries. Mucous membrane (oral/genital/perianal) TB could be either primary or secondary to autoinoculation of *Mycobacterium tuberculosis* from underlying advanced TB infection of lung, genitourinary or gastrointestinal tract respectively. Involvement of oral cavity in TB is quite rare, so difficult to get diagnosed. Hence we are reporting a case of TB of oral (buccal) mucosa in a 22 year old male who presented with painful ulcerative growth on left side of buccal mucosa near the molar teeth since 5 months. Diagnosis was made on histopathological examination and Ziehl Neelsen (ZN) staining of biopsy specimen. The complete blood count, chest X-ray, culture of tissue biopsy and sputum showed findings consistent with *Mycobacterium tuberculosis* infection. Patient responded to the treatment with complete recovery of tuberculous oral lesion. Hence awareness of rare form of oral tuberculosis makes early diagnosis and prevents further spread of disease.

Key words: *Oral Tuberculosis, Langhans Cell.*

INTRODUCTION

Tuberculosis has been the most important of human infections, in its global prevalence, devastating morbidity and massive mortality.[1.] South-East Asia carries a disproportionate 88 percent of the world's burden of TB. India accounts for nearly one-third of the global burden of TB.[2,3] The incidence of tuberculosis in the oral cavity is quite rare. It could be either primary or secondary to pulmonary disease.[4] There are about 1–1.5% cases of pulmonary tuberculosis with associated oral cavity lesions. The oral sites most frequently affected are the tongue, palate, tonsil, pharynx, and buccal mucosa.[5.] The vulnerability to TB in developing countries results from poverty, economic recession and malnutrition.[2, 3]

The epidemic of HIV infection and development of multi drug resistant bacteria also contributes to increase in number of tuberculosis cases. With increasing number of tuberculosis cases, unusual forms of the disease are also likely to increase.

Oral lesions have a non-specific presentation varying from ulcers to granulomas, fissures and may be overlooked.[4,6,7] Here we report a case of TB of oral (buccal) mucosa in 22 yr old male.

Case Report

A 22 year old male presented to the Government Dental College and Hospital Aurangabad (Maharashtra) India, with painful ulcerative swelling on left side buccal mucosa near 2nd molar tooth. The patient also had productive cough, weakness, weight loss and difficulty in opening mouth. These symptoms were gradually progressed over a period of five months. He was treated in private clinic symptomatically during this period but

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responded poorly. He had history of pulmonary TB one year back for which he had taken complete anti tuberculosis therapy (ATT) with Isoniazid 600 mg, Rifampicin 450 mg and Pyrazinamide 1500 mg, Ethambutol 1200mg for two months followed by Rifampicin and Isoniazid for four months. He had no history of dental trauma or any oral surgery or tobacco chewing.

The oral ulcer was initially small and enlarged gradually over a period of five months. Oral examination revealed tender, shallow ulcer of size 1 X 1.5 cm with irregular margins, covered with mucopurulent discharge and slough in the left retro molar trigone (figure. 1). On palpation ulcer was indurated and with undermined margins. Local examination showed diffuse swelling of left side of face. Cervical lymph nodes were neither enlarged nor fixed to surrounding tissue. Counseling was done and informed consent was obtained. Complete blood count (CBC) was normal. Erythrocyte sedimentation rate (ESR) was raised. Chest X-ray revealed widespread fibrocaseous infiltrate. The HIV antibody test was negative.

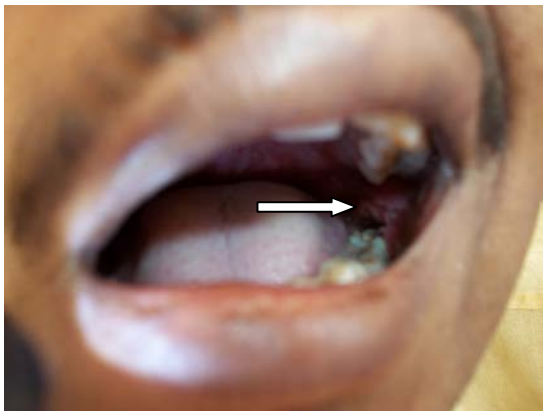

Figure 1. Oral ulcerative growth in left retro molar trigone

The patient was referred to the Microbiology and Pathology laboratory for confirmatory diagnosis. A biopsy was obtained from the margin of the lesion. Biopsy specimen was cut into tissue sections which were mounted on two glass slides. Histopathological examination with hematoxylin and eosin stain (H and E stain) of the one tissue section showed lymphocytes, Langhans type giant cells and caseous necrosis. (figure.2). Another tissue section slide stained with ZN staining showed presence of acid fast bacilli within granuloma. A culture on Lowenstein and Jensen (LJ) media from sputum and tissue lesion grew characteristic dry,

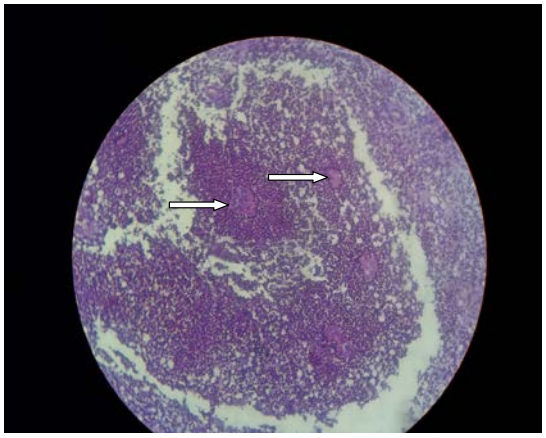

Figure 2. Histopathology (H and E stain 10x) of buccal mucosal biopsy section showing 1. Langhans type giant cell and 2. foci of caesous necrosis and plenty of lymphocytes

rough, raised and irregular colonies after 30 days. It was confirmed as *Mycobacterium tuberculosis* on ZN staining and other biochemical tests.

The patient was confirmed as a case of oral (buccal) mucosal TB with the recurrence of pulmonary TB. ATT (category II regimen) was given after consultation with TB medical officer. Category II regimen consist of Isoniazid 600 mg, Rifampicin 450 mg, Pyrizinamide 1500mg, Ethambutol 1200 mg and Streptomycin 750mg for two months then Isoniazide, Rifampicin, Pyrizinamide and Ethambutol for one month in intensive phase. This was followed by Isoniazide, Rifampicine, Ethambutol for five months. Patient's oral tuberculous lesion as well as general symptoms improved within three months.

DISCUSSION

Incidence of oral tubercular infections is quite rare, occurring in 0.05% - 5% of all tuberculosis cases.[9.] In our case, buccal mucosa is involved.

A break in the epithelial continuity facilitates inoculation by the bacilli present in sputum. Both local and systemic predisposing factors exist for the occurrence of oral lesions.[6.] Local factors include poor oral hygiene, local trauma, presence of preexisting lesions such as leukoplakia, periapical granulomas, cysts, abscesses and periodontitis.[4, 6.]

Systemic predisposing factors include primary or secondary immune suppression and nutritional deficiencies.

Orificial tuberculosis usually results from autoinoculation of the infectious agent in patients with advanced internal tuberculosis of the lungs, gastrointestinal or genitourinary tract. Haematogenous or lymphatic dissemination from another active source of tuberculosis has also been described.[9]

Most of the TB cases occurring in the oral cavity are secondary to pulmonary infections, though other primary lesions are not unknown.[4] Clinicians should also search and investigate for symptoms associated with primary TB (active source for secondary TB) which act as a source for orificial involvement.

Aird[10] has described five pathological types of oral tuberculous lesion as ulcers, tuberculoma, fissure, papilloma and cold abscess.

Shallow ulcerative growth with undermined edges was the presentation in our case.

The histopathology and culture of biopsy specimen was done for confirmation of oral (buccal) mucosal TB. The mucosal involvement was secondary to pulmonary TB.

Health care workers and the contacts of patients are at risk due to aerosol transmission. There is delay in diagnosis due to rarity of the condition. So clinician should be aware of mucosal TB and do the further investigations for favourable outcome in such cases.

CONCLUSION

A major concern about the cases of TB is the risk of transmission of tuberculosis in the community. Clinicians, especially dentists, ENT surgeons and general surgeons are involved in diagnosis and treatment of tuberculosis in rare presentation of oral mucosal TB. They are also exposed to the TB bacilli as occupational health hazard. Therefore it should be considered as differential diagnosis in chronic mucosal lesion in developing country like India. Clinicians' awareness will help in early diagnosis and prevent complications.

Acknowledgement

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The chemical nomenclature should follow the recommendations made by the recognized authority for the names of chemical compounds in Chemical Abstracts (CAS; http://www.cas.org/) and its indexes. The biochemical nomenclature should be in accordance with Biochemical Nomenclature Related Documents available at http://www.chem.qmul.ac.uk/iupac/bibliog/white.html.

The enzymes name should be used as recommended by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in Enzyme Nomenclature available at http://www.chem.qmul.ac.uk/iubmb/enzyme.

Binary names, consisting of generic name and a specific epithet (e.g. *Mycobacterium tuberculosis*) must be used for all organisms. A specific epithet must be preceded by a generic name, written out in full in its first appearance (eg. *Mycobacterium tuberculosis*) and can be abbreviated on subsequent uses (e.g. *M. tuberculosis)*.

References: The referencing style followed by the Journal is Vancouver Style. Follow the link for the reference, http:/www.library.uq.edu.au/training/citation/vacouver.pdf

Any queries related to organization and format should be addressed to editor SAARC Tuberculosis, Lung Diseases and HIV/AIDS at saarctb@mos.com.np and stac@saarctb.org.

The organization and format for submission of different kinds of manuscript are as follows.

3.2. Editorial

Editorial is written by the editorial team and is not open to the external authors.

3.3. Original article

Title page: This page should contain 1) a concise and informative title not more than 125 characters (including spaces) in bold faced upper case letters and without abbreviations 2) Names and affiliations of all contributing authors in bold faced letters, place an asterisks as a superscript for a corresponding author 3) The full name of corresponding author, designation, affiliation, address, single e-mail should be provided. This will be published in the article to facilitate communication 4) word count of text (not more than 3000 words) excluding titles, references, tables and figures.

Abstract: Should be written in structured format (Introduction, Methodology, Results and Conclusion) and should not be more than 250 words excluding the titles. Objectives should be the last sentence of the introduction. Do not write the experimental details. The abstract must be understandable without referring the text. Avoid abbreviations and references. Do not include tables and figures.

Key words: Below the abstract identify 3-5 key words to assist indexers in cross-indexing the article. Non-standard abbreviations should be avoided. First letter of each key word should be written in upper case. All the key words should be italicized.

Introduction: The introduction should be sufficient to provide the background information to allow reader to understand the hypothesis and rationale for the study without referring to other publications in the topic. Most appropriate references should be selected to provide most salient introduction rather than explicit review of the topic. Explain the abbreviation at its first appearance

Methodology: This should include sufficient information including study design, setting, study period, study population, selection of subjects (inclusion and exclusion criteria), scientific basis of selection of sample size, method of sampling, data collection procedures in detail, ethical consideration, data analysis and statistical tools used. The information on source of materials (name and location of manufacturer) must be provided. If numerous methodologies already exist, brief explanation of the procedure and the reference is sufficient. If the procedure is new, all technical details of the procedures should be written. This is to allow the study to be repeated by others. Statistical analysis if any should be mentioned in this section.

Results: Theresult should be presented in a sequential manner in text, tables and figures as concise as possible. Avoid using extensive graphs, tables and figures which can be written in text. Make sure they are all numbered in the order they appear in the text. Whatever has been presented in the table and figure need not to be written in text.

Discussion: This section must not extensively repeat the results instead should provide an interpretation of the results in relation to previously published work. The implications of the findings, their limitation and recommendations should be presented. Avoid unqualified statements and conclusions which are not completely supported by data. Avoid claiming priority. New hypothesis may be labeled as recommendations.

Conclusion (s): Summarize your findings and highlight the importance of the study. Simply do not repeat what has already been mentioned in previous sections of the manuscript. Based on the findings a recommendation should be made.

Acknowledgement (s): The source of any financial support for the work being published must be indicated in this section. Recognition to any personal assistance should also be mentioned in this section. The authors also need to declare financial or competing interest if any.

References: The referencing style followed by the Journal is Vancouver Style. Follow the link for the reference http:/ www.library.uq.edu.au/training/citation/vacouver.pdf

3.4. Review/Minireview

Reviews should not merely be the collection of previous findings in quotes from journals, reports and text books. It should be up to date, accurate and should contribute significantly to the scientific community. The review should be in depth analysis of the problem, background to this problem, science behind the problem, methodology, discussion, recommendation, conclusion, future perspectives, acknowledgement and references. Abstract should be unformatted and not more than 300 words and the text should not be more than 4500 words. The tables and figures (combined) should not be more than 7. The references should not be more than 40.

The Minireviews should be focused discussions of defined topics relevant to the scope of the SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS. They are not expected to be comprehensive reviews of the literature but rather focused discussions of specific topics. The minireview should include analysis of the problem, background to this problem, science behind the problem, methodology, discussion, recommendation, conclusion, future perspectives, acknowledgement and references. A standard title page should be provided. This is followed by an unformatted abstract which should be not more than 250 words and then the text of the minireview should not be more than 3500. Up to 5 tables, figures, or photographs (combined) may be included. Less than 30 references should be used. Minireviews will be reviewed by the SAARC Tuberculosis, Lung Diseases and HIB/AIDS editors and will be peer reviewed.

3.5. Case reports

AA Case Report should include five sections; abstract, introduction, case report, discussion and conclusion. The title page must include title, authors list and their affiliations and corresponding author's name, affiliation and address. The abstract should be no more than 150 words. The abstract should be structured and should include introduction, patient, result and conclusion. The abstract should follow by key words, 3-5 key words. The body of case report should not be more than 1000 words and should include introduction, case report, discussion and conclusion. This should be followed by acknowledgement and references (not more than 10). The total number of tables and figures (combined) must not exceed 2.

3.6. Letters to editors

Letters to editor should not be more than 500 words and must cite references (not more than 7) to support the writer's argument. For Letters commenting on published articles, the cover letter should state the volume and issue in which the article was published, the title of the article, and the last name of the first author. Letters to the Editor do not have abstracts.

3.7. Short communication

The short communications that are within the scope and are of particular interest to the readers of the SAARC Tuberculosis, Lung Diseases and HIV/AIDS are published. Abstract should be no more than 150 words. Manuscripts are limited to 1000 words, one figure, one table and not more than 10 references.

3.8. Errata

This section provides an opportunity of correcting errors that occurred during the writing, typing, editing, or publication. These errors could be a misspelling, a dropped word or line, or mislabeling in a figure in a published article. Authors can submit errata using the online manuscript submission or via the email (See below).

4. Submitting manuscript

Manuscripts can be submitted online (www.saarctb.org) or through email to the Chief Editor, SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS. Authors should ensure following documents to be sent if he/she wishes to send manuscript via email or online system. 1) Cover letter 2) Authorship form 3) Declaration form 4) Manuscript (Title page, Abstract, Body of article, References) and 5) Letter of Ethical Approval or A statement of clearance of the study protocol and the study by the Ethical Committee/Board mentioned in Methodology.

5. Publication charge

The SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS is available in printable and online open access electronic versions and is free of charge.

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