Original Paper

Leishmaniasis amongst TB patients from Kala-azar endemic areas admitted to Surya Kanto Hospital, Mymensingh

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Abstract

Introduction: Parasitic diseases and Tuberculosis were shown to be the risk factor for each other. Co-infection of visceral leishmaniasis and pulmonary tuberculosis are increasing public health problem now a days especially in developing countries. More than 81% of cases and deaths from TB are in developing countries and is aggravated by concurrency with parasitic diseases.

Objective: To find out the occurrence of Leishmaniasis amongst Tuberculosis (TB) patients of Kala-azar endemic areas admitted to Surya Kanto (SK) Hospital, Mymensingh.

Materials and Methods: This descriptive cross-sectional study was conducted at Infectious and Tropical Disease Department of Mymensingh Medical College Hospital, a tertiary referral hospital from January 2014 to Decmber 2016. A total 176 TB patients reported from Kala-azar endemic areas admitted were included. For confirmation of diagnosis all cases of TB were sent for Gene expert before that relevant tests like CBC (Complete Blood Count), X-ray chest, Mantoux test, Smear tests were done. After having confirmed all the TB cases were investigated for Kala-azar. Venous blood was collected for buffy coat and serum for ELISA. All 176 patients' blood were tested with ICT(rK39) for VL. There after splenic aspiration were done with full aseptic preparation for only those who were ICT positive. Smears were prepared and viewed under microscope with 10x 100 magnification. After that all these cases were tested by ELISA.

Results: A total of 176 TB patients were selected in this study of which 120(68.1%) were male and male-female ratio was 2.1: 1, the mean age was 43.06 years. Among patients 87.49% were within 16 to 60 years of age that means within productive age of life are infected more with TB. In terms of education 79.10%(139) patients were below Secondary level, that revealed the illiterate and or less literate people were affected more by TB. Maximum patient's family 132(74.99%) had monthly income up to 10,000.00 BDT (125 US$)i.e. the poorer people of the area. Out of 176 patients Smear positive TB cases were 170(97.41%) but By Gene Xpert all 176 patients were found positive. The blood sample of 176 patients were tested with ICT rK39 and 12(6.81%) patients were found positive for leishmaniasis who suffered from TB. In splenic smear test 12 patients smear were examined of which 11(91.66%) were positive but by ELISA all 12(100%) were found positive.

Conclusion: Visceral leishmaniasis and tuberculosis co-infection have drawn attention to the clinical aspect. In both diseases infection may remain dormant asymptomatic which may be related to immune suppression and may lead to active disease. This study has been able to find out leishmaniasis amongst tuberculosis patients from kala-azar endemic areas of Bangladesh.

Key-words: Leishmaniasis, TB patients, endemic areas, Surya Kanto (SK) Hospital, Splenic aspiration, GeneXpert, ELISA, Smear positive.

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Introduction

Kala-azar (Visceral Leishmaniasis) is a disease which blights the developing world with 200,000 to 400,000 new cases and 48,000 deaths annually. The vast majority of cases are seen in six countries-Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan. There are no vaccines available and current drug treatments all have limitations of prolonged administration mainly by injection, high cost, drug resistance, toxicity and potential for foetal malformations etc[1].

Parasitic diseases and Tuberculosis (TB) were shown to be the risk factors for each other[2]. Co-infection of Visceral leishmaniasis (VL) and pulmonary tuberculosis are increasing public health problem especially in developing country[3]. And both are major public health problem in Bangladesh. More than 81% of TB cases and deaths come from developing countries; the TB situations in those countries is aggravated by high prevalence of HIV, concurrency with parasitic diseases, drug resistance, social inequalities, poor TB control efforts and inadequate health care spending[4].

Diagnosis of TB is mainly based on microscopic detection of acid fast bacilli in smear which has 40 to 70% Sensitivity and Culture which needs up to 8 weeks' time[5]. Negative sputum smear does not exclude TB in highly suspected cases[6]. Among TB patients, prevalence of parasitic disease varies widely in different areas and different survey sites. In Sudan, up to 77% of TB patients were positive for the leishmania skin test in the community[2].

The co-infection of TB and parasitic diseases have been reported in many studies for almost past 70 years, although great achievements have been gained in the fields of TB and parasitic disease control and prevention respectively[7]. Some studies showed that the immune response was modified in the co-infection situation[8]. Bangladesh experiences dual epidemics of TB and VL[9].

TB-VL co-infection is a syndrome that has important clinical implications. Although distinct in etiology and transmission mechanisms, VL and TB share several features and many infections remain asymptomatic. Symptoms usually develop after several months or years in those who progress to clinical disease; very long incubation periods (latent infection) may be related to immune suppression occurring at a later age, which may activate latent infection to active disease[10]. Co-infection of pulmonary tuberculosis and Visceral leishmaniasis are increasing public health problem in eastern region of India. Such type of co-infections leads to decreased host's immune system[11].

This study was designed to find out the occurrence of Kala-azar amongst the TB patients admitted to Surya Kanto (SK) Hospital, Mymensingh Medical College Hospital from Kala-azar endemic areas of Mymensingh and Pabna district.

Materials and Methods

This was a descriptive cross-sectional study conducted at Infectious and Tropical Disease Department, SK Hospital of Mymensingh Medical College Hospital (MMCH) from January 2014 to December 2016. MMCH is a tertiary referral teaching hospital in greater Mymensingh area in the northern part of Bangladesh covering almost 25 million people. From reported patients (referred and direct) 176 TB patients from Kala-azar endemic area of Trishal, Fulbaria and Madhupur Upozilla (Sub district) and other TB patients from kala-azar endemic area of Chatmohor Upozilla, Pabna district admitted to SK Hospital were included. TB patients admitted in the hospital from other places were excluded. These patients initially diagnosed as TB from respective Health Complexes and in outpatient settings of SK Hospital were admitted. For the purpose of this study all the TB cases includinghighly clinically suspected negative cases (admitted) were sent to Mymensingh TB and Leprosy Control Project (MTLCP), Shambhuganj, Mymensingh for GeneXpert to confirm diagnosis. As such one hundred and Seventy six patients were sent for GeneXpert. The GeneXpert is a cartridge based nucleic acid amplification test which can identify Mycobacterium tuberculosis DNA and resistance to rifampicin. Some organisations have claimed that the GeneXpert test is going to revolutionize the diagnosis and care of people with TB[12]. Routine Laboratory tests like Complete blood count, X-ray, Mantoux test, Smear tests from cough, or other exudates for Mycobacterium were done as per the hand book of Stop TB Partnership, Global edition 2013[13]. Two sputum smears for each patient were done and examined under Fluorescence microscope following National Guidelines and Operation Manual for Tuberculosis control 2013[13]. After having confirmed all the TB cases were investigated for VL (Visceral Leishmaniasis) with Immuno Chromatographic Test (rK39), smear microscopy from splenic aspiration and ELISA.

Five ml venous blood of each patient was collected of which 3 ml was transferred into vacutainer containing anticoagulant, centrifuged for buffy coat and 2 ml to a sterile test tube for serum preparation. One drop of blood was applied to the base of rK39 strips (Kala-azar DetectTM, InBiosInc, USA) impregnated with recombinant rK39 antigen. After air dried 3 drops of test buffer was added to the strips kept upright. Strips were observed after

10 minutes and the appearance of lower red band(Control) and upper red band indicated presence of anti rK39 IgG signifying a positive result.

After full aseptic preparation splenic aspiration was performed by a well experienced doctor of SK Hospital on all the study patients (176 TB patients) suspected as VL. Two smears were prepared for microscopic examination. After being dried in air, smears were stained with leishman stain and was examined under 10X100 magnification for visualizing the parasites. Smears were graded as Positive (+) and Negative (-) in the presence and absence of parasites respectively.

ELISA (Enzyme Linked Immunosorbent Assay) was carried out for serum of 12 patients whose splenic aspirates were positive for VL. The tests were performed in flat-bottom 96 microtitre plates (Maxi-Sorp; Nunc, Rosekilde, Denmark). The plates were coated with 5µg of crude or acetone treated antigen per well in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and incubated overnight at 4°C. Positive control used from patient's serum who had VL and was admitted in SK Hospital and negative control was used from serum of healthy persons from non-endemic areas. After being blocked with casein buffer (1% casein in 0.05 M Tris-HCl buffer with 0.15 M NaCl, pH 7.6) for 2 hours at room temperature, the wells were loaded with 100 µl of serum (1: 4,000dilutions in casein buffer) and incubated at 37°C for 1 hour. The plates were washed four times with PBS (Phosphate buffered saline) containing 0.05% Tween 20 (pH 7.4) and incubated with peroxidase-conjugated goat anti-human IgG (Tago, Camarillo, Calif.), IgA (Zymed, South San Francisco, Calif.), or IgM (Tago) (1: 4,000 dilutions in casein buffer) at 37°C for 1 hour. After being washed four times, the plates were incubated with substrate ABTS (KPL Inc., Gaithersburg, Md.) for 1 hour at room temperature. Colourappeared,comparing with control result is observed as positive and negative. Datawere compiled on the table and analyzed by SPSS programme.

Results

During the period from January 2014 to December 2016 a total of 176 TB patients were selected in this study. Among the study population 120(68.1%) were male and 56(31.8%) were female. The male: female ratio of TB patients was 2.1:1 with the mean age 43.06 years and Median 39.46 years. Almost eighty eight (87.49) percent of patients both male and female were found within 16-60 years of age i.e.in the productive age of life (Table- I).

Table-I: Age and sex distribution of TB Patients (n=176)

Age group (years)	Male	Female	Total	%
0 - 15	07	03	10	5.68
16 - 30	29	15	44	25.00
31 - 45	42	18	60	34.09
46 - 60	33	17	50	28.40
61 - 75	06	02	08	4.54
76 and above	03	01	04	2.27
Total	120(68.18%)	56(31.82%)	176	100

From Table-II it is evident that 91% people were educated below Secondary level education, it reveals the illiterate and or less literate people were affected more by TB.

Table-II: Distribution of patients by literacy and area they belong (n=176)

Area of respondents	Illeterate	Class-V	Class-VIII	SSC Pass	HSC and above	Total
Fulbaria	25 (43.9%)	09 (15.8%)	10 (17.5%)	06 (10.5%)	07 (12.3%)	57 (32.4%)
Trishal	14 (29.2%)	15 (31.2%)	9 (18.8%)	07 (14.6%)	03 (6.2%)	48 (27.3%)
Modhupur	16 (34.8%)	13 (28.3%)	10 (21.7%)	04 (8.7%)	03 (6.5%)	46 (26.1%)
Chatmohor	06 (24.0%)	05 (20.0%)	07 (28.0%)	04 (16.0%)	03 (12.0%)	25 (14.2%)
Total	61 (34.7%)	42 (23.9%)	36 (20.5%)	21 (11.9%)	16 (9.1%)	100 (100%)

Note: As per Bangladesh Bureau of Statistic 2016; Literacy rate at Fulbaria 25.23%, Trishal 40.2%, Modhupur 41.2% and Chatmohor 43.4%

Regarding monthly family income maximum patients 132 (74.99%) had up to 10,000.00 BDT (125 US$) i.e. the poorer people of the area (Table-III).

Table-III: Distribution of Patients by monthly family income (n=176)

Monthly family income	Number of Population	%
Up to 5000 BDT	83	47.41%
5000 to 10000	49	27.58%
10000 to 15000	23	12.93%
15000 to 20000	12	6.89%
Above 20000	09	5.17%
Total	176	100%

Out of 176 initially diagnosed TB patients a total of 170 (96.59%) were found positive (Table-IV).

Table-IV: Distribution of Patients by smear microscopy

	Male	Female	Total
Patients	119	57	176
Smear TB+ve cases	116	55	170
Positivity rate	97.48%	96.49%	96.59%

A total of 176 patients were sent for confirmation of diagnosis for GeneXpert to MTLCP Shambhuganj, Mymensingh and all 176 were found positive for TB of which 22 were found Multi Drug Resistant (MDR) (Table-V).

Table-V: Patient sent for GeneXpert

Patients sent for Gene Xpert		Positive for TB	MDR
Male	119	119	12
Female	57	57	10
Total	176	176	22

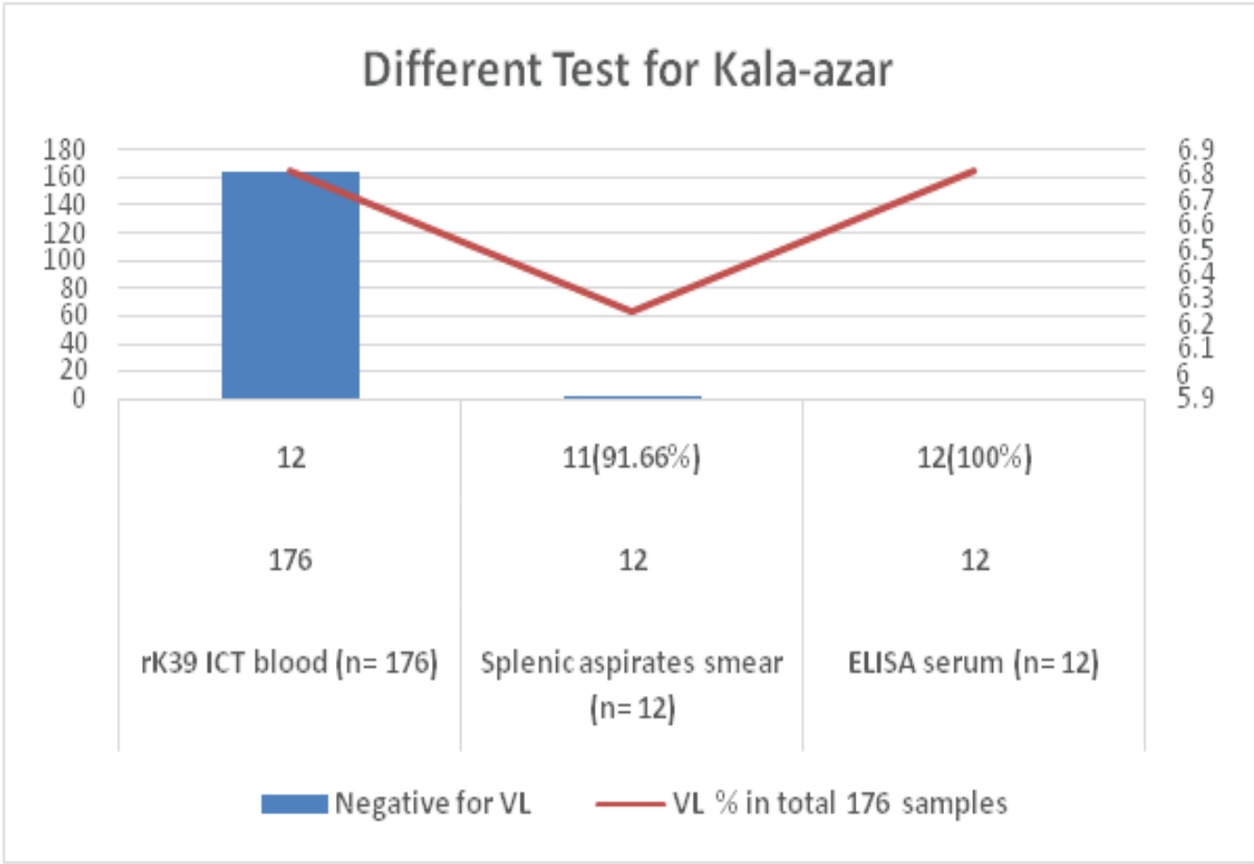

Fig-1: Distribution of patients by different tests for Kala-azar

Discussion

In this study atotal of 176 TB patientswere included during the period of January 2014 to December 2016, the mean age was found 43.06 years, the male-female ratio was found 2.1:1. In the study Jermiah Seni et al the mean age was found 36.1 and male-female ratio was 1.17:1. Maybe it was due to higher HIV positivity rate where male femaleis equally affected and lesser in elderly population[14]. In the study of Takele Tedesser et al, the mean age was found 34 years and male female ratio was 0.9:1 where it might be due to two thirds of the symptomatic sputum smear positive tuberculosis remains undiagnosed and higher HIV prevalence rate of fourto six times[15]. In the present study majority TB patients were found in productive year of life 16-60 years.

Literacy plays an important role in making awareness of the people and thereby develop preventive knowledge of the people. In this study 160(91%) respondents were found to have below secondary level of education which is really critical for Tuberculosis. It delineates that illiterate and or less literate people are affected more by TB. In a study Mondal MNI[16] it was found that 85.9% of respondent had below 12 years of schooling i.e. below higher secondary level. The persons with higher level of education scored better on TB knowledge indices than those with less education or those who were illiterate[17].

Illiteracy and poverty equally associated with tuberculosis. In the present study, 74.99% patients had monthly family income up to 10,000.00 BDT (125 US$) i.e. the poorer people of the study area and are affected more by tuberculosis. Irfan SD et al found in their study that 61% respondents had monthly income less than 25,000.00 BDT and the respondents having higher monthly income (>25000.00 BDT) were less susceptible to tuberculosis. This study was conducted in Dhaka city (Shyamoli TB Hospital) as such income was higher[18]. In another study by Raza AKMM found 69.6% respondents had annual family income up to 1,50,000.00 BDT (Monthly income 12,500.00 BDT) and they mentioned tuberculosis affected middle class and lower class economic population group[19].

In this study smear positive results were found in 170 (97.41%) of all initially diagnosed TB patients where patients were not the suspects but selected. This positivity rate was much higher to the recommended rate 10% of IUATLD (International Union Against Tuberculosis and Lung Diseases)[20]. In a study by Horie T et al in Hanoi, Vietnam the smear positive case finding was 3.3 times higher for females than males and was due to the active case finding via household visits that reduces the social inequity in accessing health services[21]. Yassin and Cuevass in their study found that the smear positivity rate (two or more positive smears) was 25%, with a range of 16.8–36.4% per zone. This exceeds the international recommendations of examining 10 suspects to identify one case[22].

This study confirmed 176 initially diagnosed TB of patients by Gene Xpertand also 22 (12.5%) MDR TB during the study. In Aurin T H et al study among 300 samples, 191 (63.7%) and 193(64.3%) cases were found to be resistant against rifampicin in LPA (line prob assay) and GeneXpert methods, respectively[23]. Despite success in tuberculosis control, multi-drug resistant tuberculosis in Bangladesh is increasing and currently multi-drug-resistant tuberculosis rate is 3.6% in new cases and 19% in re-treatment cases[24].

After having confirmed the presence of TB of all 176 patients, serum was tested by Immuno Chromatographic test (ICT) by rk39 dipstick strip. It was found 12(6.8%) patients were positive for Visceral Leishmaniasis. Rahi AA in Iraq found 62% positive cases by rk39 dipstick test[25]. In another study done in Southern Europe, the strip test was positive in 71.4% cases[26]. In Khan MGM it was mentioned that out of 25 non–symptomatic control individual from an endemic region 04(16%) were found positive for VL[27]. The ICT based rK39 antibody test used in this study diagnosed VL and has been widely used in Bangladesh.

Splenic aspiration was done on those 12 patients who were ICT positive and theirsmears were seen under microscope in which 11(91.66%) were found positive for Visceral leishmaniasis; that revealed 11 samples were found positive for VL out of total 176 samples i.e 6.25% from total samples. In Khan MGM, all suspected cases of VL were found 100% (n= 61) positive[27]. In another study by Shamsuzzaman AKM, it was revealed that splenic aspiration was done for five patients and smear was examined under microscope for VL and all 05(100%) were found positive[28].

For more confirmation, these 12 patient's serum were tested with ELISA in which 12(100%) became positive; and calculating with total samples 176 it was 6.8% positive. In Islam MZ, 40 patient's serum were tested for ELISA and 36(90%) were found positive[29]. In Islam MZ, it was seen that out of 585 individuals 40(6.8%) were found positive[30]. In another study by Podder MP, it was stated that out of 50 patients 27(54%) were found positive[31]. In this study, it is found out that 6.8% patients from kala-azar endemic areas had both TB and kala-azar. TB is an immunosuppressive condition that progresses latent leishmaniasis and results in clinical leishmaniasis and visceral leishmaniasis can reactivate latent tuberculosis[32].

Conclusion

This study has been able to find out leishmaniasis amongst tuberculosis patients from kala-azar endemic areas of Bangladesh. Visceral leishmaniasis and tuberculosis co-infection poses impact clinically. These diseases have clear epidemiological parameters in causation, transmission and host behavior. But sometimes in certain counts shares some features and may incur many infections to be dormant and asymptomatic that may lead to immune suppression which in turn activate latent infection to active disease. Patient getting anti TB continuously but is not improving then after having investigated found VL that is to be kept in the mind of the treating physician and is unique in kala-azar endemic areas. About 7% leishmaniasis cases were found in tuberculosis patients in this study which may be a tip of iceberg and warrants further elaborate study.

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