Diagnosis and Antimicrobial Susceptibility of Mycobacterium Tuberculosis in a Tertiary Care Hospital in Bangladesh

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Abstract

Background: Tuberculosis (TB) is a common condition all over the world but more common in developing countries like Bangladesh38. This is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. In 2013, 9 million people fell ill with TB and 1.5 million died from the disease globally. Approximately 15-20 % of total TB cases are extrapulmonary in non-HIV patients. Among extrapulmonary TB, abdominal tuberculosis (ATB) accounts for 11%-16%. Abdominal TB is difficult to diagnose because of its lack of specific symptoms, low yield of acid-fast bacilli (AFB) on smear and culture due to paucibacillary lesion; and variable manifestations depending upon anatomical localization of the disease. The diagnosis of abdominal TB classically requires histopathological, microbiological and culture confirmation of Mycobacterium tuberculosis. Investigations like Imaging (Ultrasound, Barium X-Rays, and CT scan) and the Mantoux test have only supportive value. Aim of the study: The aim of the study was to diagnose modalities and antimicrobial susceptibility in abdominal tuberculosis patients. **Material & Methods:** This cross-sectional hospital-based observational study was carried out among 73 adult patients with the diagnosis of abdominal TB who met the selection criteria and attended in Gastroenterology department of DMCH through either admission or referral from May 2015 to April 2016. **Results:** The most frequent symptoms were weight loss (96.9%), abdominal pain (75%), and fever (75%). The most frequent signs were anaemia (34.4%), followed by ascites (27.9%). Basis of diagnosis in abdominal tuberculosis was histolopathogy in 37.5%, Gene Xpert in 28.1%, Positive AFB on culture in 9.4%, ADA value in ascitic fluid (Cutoff >40 IU/L) in 25%, and good clinical response (Based on weight gain and general improvement in wellbeing) to a therapeutic trial of anti-TB treatment in 18.7% patients. Drug sensitivity pattern was analyzed in all three AFB culture-positive patients; resistance was detected in one which showed multidrug resistance (MDR TB). **Conclusions:** The result of this study highlighted the diagnostic yield of various investigation modalities, particularly newer modalities (Gene Xpert, culture sensitivity in Bactec MGIT 960) and basis of diagnosis in abdominal TB. This study also determined the MTB culture positivity from tissue biopsies in patients with abdominal TB and demonstrated drug-resistant MTB in culture-confirmed abdominal TB.

INTRODUCTION

Tuberculosis is a disease that has plagued humanity for millennia. There are an estimated 7.5 million cases of tuberculosis in the global population, with 2.5 million fatalities every year, making tuberculosis the leading cause of infection-related death behind HIV/AIDS.[1] Antimicrobial resistance in Mycobacterium tuberculosis, both primary and acquired, is a worldwide concern.[2,3,4] It is still a serious public health issue in Bangladesh,[1] and the rapid growth of drug-resistant mycobacteria has increased the necessity for quick detection and efficient treatment. MDR (multidrug-resistant) strains are becoming more common. Laboratories must provide prompt diagnosis and efficient antimicrobial susceptibility testing (AST) for effective illness treatment.[5,6] Though the BACTEC 460TB technique (Becton Dickinson Co., Towson, MD) is a well-established, semi-automated, broth-based method for fast detecting mycobacteria within a closed system, this system detects mycobacterial growth using a radiometric method. The disposal of radioactive waste generated by the BACTEC 460TB method presents a significant logistical challenge and increased costs.[7] The essential medications in treating tuberculosis are rifampin (RIF) and isoniazid (INH), and resistance to these antibiotics often incurable tuberculosis.[8] Due to the lengthy turnaround time for standard susceptibility testing, patients infected with drug-resistant tuberculosis may receive poor treatment, allowing resistant strains to spread.[9] It takes 6-9 weeks to diagnose tuberculosis in the lab and then test the isolated bacterium for treatment resistance using standard procedures.[10,11,12] Simultaneously, quick drug resistance evaluation is critical for selecting effective pharmacological therapy and avoiding the spread of drug-resistant strains. As a result, a tuberculosis control program faces the issue of developing new quick methods for evaluating medication resistance. Mutations in an 81-bp region of the rpoB gene encoding the beta subunit of RNA polymerase (rifampin resistance-determining region [RRDR]) cause RIF resistance in Mycobacterium tuberculosis strains collected around the world.[13,14,15] Early detection and treatment of patients and contacts are critical for the success of control strategies. Patients may fail to respond to anti-tubercular therapy for various reasons, one of which is medication resistance, which is becoming increasingly common in both developed and developing countries.[16,17,18,19] Different methods for assessing drug susceptibility of tubercle bacilli have been employed in the past, with the proportion approach using Lowenstein-Jensen (L-J) medium being the most frequently acknowledged.[20] E test, bioluminescence, polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP), and other nontraditional procedures are utilized in many laboratories. Non-uniformity and lack of standardization of laboratory processes, which is also a significant issue, may impede the accurate assessment of drug resistance, resulting in misleading data and hence a lack of understanding of the true incidence of drug resistance.[21] It would be ideal to have a simple, quick, and consistent

approach for determining drug susceptibility that does not require expensive equipment. Antimicrobial resistance must be detected quickly if the spread of resistant Mycobacterium TB is to be controlled. The results of various inquiry methodologies for tuberculosis diagnosis vary.[22] Smear and culture yields of organisms in the ascitic fluid are minimal. In less than 3% of cases, acid fast bacilli staining is positive. In less than 20% of cases, a positive culture is obtained.[23] The activity of adenosine deaminase (ADA) in ascitic fluid is a sensitive and specific indicator of tuberculosis.[24] Because ADA is found in T-lymphocytes and macrophages, its levels rise in response to T-lymphocyte stimulation by CMI to mycobacterial antigens.[25] According to a study, sensitivity was 100 percent, and specificity was 96 percent when the cutoff value of 40U/L was used.[1] Endoscopic biopsy has a diagnostic yield of between 30 and 80 percent >.[26] In a study of 61 patients with abdominal TB in Mumbai, India, histology was used to diagnose 78.6% of the cases.[27] In a study of 209 patients with abdominal TB in Pakistan, histopathology was analyzed in 42% of the cases.[22]

Objectives

To diagnose the suspected cases of abdominal tuberculosis patients.

MATERIAL AND METHODS

Type of study

This is an observational prospective clinical study.

Place and period of study

This study was conducted in the Gastroenterology department of Dhaka Medical College Hospital (DMCH) in collaboration with National Tuberculosis Reference Laboratory, NIDCH, Mohakhali, Dhaka from May 2015 to April 2016.

Study population

Patients diagnosed with abdominal tuberculosis (based on positive AFB culture, Gene Xpert, histopathology, ascitic fluid ADA report, and response to anti-TB trial) attended in the Gastroenterology department of DMCH through admission or referral.

Inclusion criteria

- Abdominal tuberculosis patients
- Admitted or referred patients
- Age-any adult patient

Sample selection

A total of 73 patients who attended the Gastroenterology department of DMCH either through admission or referral for May 2015 to April 2016were selected considering the inclusion criteria.

Statistical data analysis

The statistical analysis was performed using MS Excel 2016 and statistical package for social sciences (SPSS) version 25.0 for Windows (SPSS, Chicago IL, USA). The data of categorical variables are presented as frequencies and percentages.

RESULTS

Table 1 shows the frequency distribution of the signs and symptomsof abdominal tuberculosis in a Tertiary Care Hospital in Bangladesh with a percentage rate. According to patients' data, most people lost their weight, close to 97%. Maximum patients (75%) had abdominal pain and severe fever symptoms. Loss of appetite (68.8%) and night sweats (34.4%) aresignificant symptoms of the people of this research. One-fourth of the respondents (25%) had diarrhoea, alteration of bowel habits, and constipation.Only a tiny proportion of the patients faced vomiting, and the amount was 15.6%.

This distributive table also indicates the most frequent signs of abdominal tuberculosis patients. Maximum patients had the problems of anemia (35%), ascites (27.9%), and mass in the right lower quadrant (18.8%). A few of the respondents encountered abdominal lymphadenopathy (9.3%), oedema (6.3%), and hepatomegaly (6.3%).

Table 1: Most frequent signs and symptoms of abdominal tuberculosis in a Tertiary Care Hospital in Bangladesh

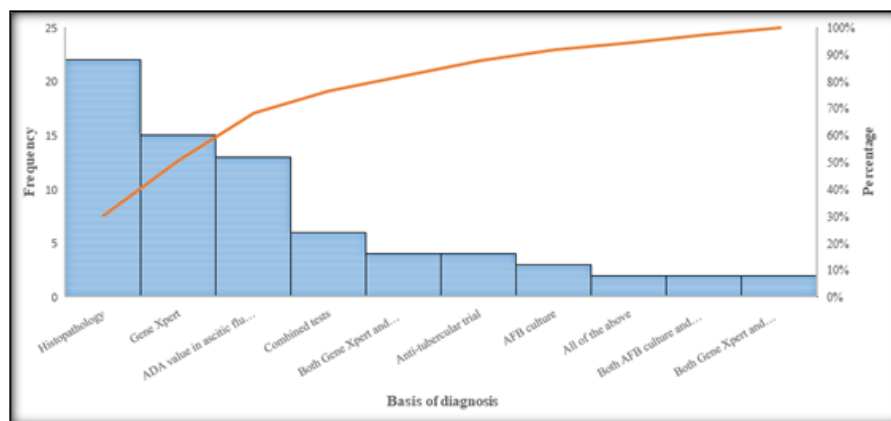| Symptoms	Frequency	Percentage	Signs	Frequency	Percentage
Weight loss	70	96.9%	Anemia	25	34.4%
Abdominal pain and fever	54	75%	Ascites	20	27.9%
Loss of appetite	50	68.8%	Mass in the right lower quadrant	14	18.8%
Night sweats	25	34.4%	Distension of abdomen	9	12.6%
Diarrhoea and bowel habit change	18	25%	Abdominal lymphadenopathy	7	9.3%
Constipation	18	25%	Oedema	5	6.3%
Vomiting	12	15.6%	Hepatomegaly	5	6.3%

Figure 1: Basis of diagnosis of the study patients in abdominal tuberculosis in Bangladesh

[Figure 1] illustrates the basis of diagnosis in abdominal tuberculosis respondents in a tertiary care hospital in Bangladesh. Histolopathogy showed epithelioid cell granulomas with or without caseation in the maximum amount of patients (22) as well as Gene Xpert of tissue sample showed MTB DNA and ADA value in ascetic fluid (Cutoff >40 IU/L) exposedin 15 and 13 people, respectively. Besides this, positive AFB on the culture of tissue sample was found in 3 patients who reported undergoing drug susceptibility testing, two isolates were susceptible to all drugs (pan-susceptible), and only one showed resistance which is multidrug resistance (MDR TB).

DISCUSSION

Abdominal TB is a complex disease and has diverse symptomatology that is non-specific.[22] The most frequent presenting symptoms in this study were weight loss (96.9%), abdominal pain (75%), fever (75%), loss of appetite (68.8%), night sweats (34.4%). This is relatively equal as compared to a previous study where the most frequent symptoms were abdominal pain (93%), fever (64%), night sweats (48%), and weight loss (47%).[22] Also, coinciding with the study result, abdominal pain and weight loss appeared to be the most frequent symptoms.[28] Moreover, the most frequent signs were anaemia (34.4%), ascites (27.9%), mass in the right lower quadrant (18.8%), distension of the abdomen (12.6%), abdominal lymphadenopathy (9.3%), Oedema (6.3%) and hepatomegaly (6.3%). A study showed anemia in 70.4%, ascites in 38.4%, and mass in the right lower quadrant in 3.2%of patients.[29] This current study also shows the basis of diagnosis in abdominal tuberculosis were follows:

histolopathogy showed epithelioid cell granulomas with or without caseation in the maximum amount of patients (22) as well as Gene Xpert of tissue sample showed MTB DNA and ADA value in ascitic fluid (Cutoff >40 IU/L) exposedin 15 and 13 people respectively. Besides these, good clinical response (based on weight gain and general improvement in wellbeing) to therapeutic trial anti-TB treatment in 4 patients. 6 patients were diagnosed based onthe combination of various tests (Histopathology, Gene Xpert, AFB culture, and ADA value in ascitic fluid). Among them, the basis of diagnosis of both Gene Xpert and histopathology were in only 4 patients, both AFB culture and histopathology were in just 2 patients, both Gene Xpert and ADA value in the ascitic fluid were in 2(2.74%) patients, and combination of all tests (Histopathology, Gene Xpert, AFB culture and ADA value in ascitic fluid) was in 6 (8.22%) patient. Basis of diagnosis in abdominal tuberculosis in a previous study were: histolopathogy showed epithelioid cell granulomas with or without caseation in 42% patients, Positive AFB on the culture ofa tissue sample in 2.9% patients, and good AFB on the culture of tissue sample clinical response to a therapeutic trial of anti-TB treatment in 2.3% patients.[22] Another study showed that 8.1% of patients responded positively to the anti-tubercular test.[27] In another literature, up to 40% of patients were given a therapeutic trial of anti-tuberculosis drugs.[30] Furthermore, positive AFB on the culture of tissue sample found in 3 patients report undergoing drug susceptibility testing. Two isolates were susceptible to all drugs (pan-susceptible), and only one showed resistance, which is multidrug resistance (MDR-TB).A similar study showed that 16.39% of isolates

were susceptible to all drugs (pan-susceptible), and 5.4% of patients had MDR-TB.[27] Another similar type study showed 13% of patients had MDR-TB.[31]

CONCLUSIONS

Tuberculosis is a global public health issue that will continue to gain attention, owing to the AIDS pandemic and a lack of new effective treatments. Because of its drug resistance, it has become a global issue. In addition, Mycobacterium tuberculosis strains resistant to one or more first-line therapies are becoming increasingly common, resulting in high-mortality outbreaks.[32,33,34,35,36] These issues highlight the necessity for speedy and accurate techniques for detecting resistant species. The availability of data on the gene alterations that causes antibiotic resistance will aid in achieving these objectives. Molecular

approaches have begun to transform drug susceptibility testing and identification by increasing sensitivity and speed. According to various studies, abdominal TB was usually identified in the third to a fourth decade, with a male majority, and had diverse and vague symptomatology. In all patients, no one test was sufficient to diagnose abdominal tuberculosis. For the diagnosis of ATB, a high index of clinical suspicion was necessary and the use of numerous adjuvant diagnostic methods. For the diagnosis of ATB, the diagnostic yield of several investigation methods was variable. The growth of Mycobacterium tuberculosis is an issue in both industrialized and developing countries, such as Bangladesh. There are few studies on abdominal TB in Bangladesh, and most of them are small case series. However, in Bangladesh, data on MDR-TB in pulmonary TB is available, but no data on MDR-TB in abdominal TB has been published.

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