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STUDY ON THE CURRENT STATUS OF SECONDARY INFECTION OF PULMONARY TUBERCULOSIS PATIENTS IN BANGLADESH

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ABSTRACT

Tuberculosis (TB) is one of the most highly infectious disease in Bangladesh and secondary bacterial infection along with TB may delay the curing period of tuberculosis resulting in arises of various complication like Multi Drug Resistance (MDR). In present study, a total of 450 TB suspected patients were examined during September to December 2012 period. Among those, 100 samples were cultured for isolating secondary bacterial infection of newly detected pulmonary TB (PTB) patients whose were already treated by TB drugs. From these culture samples, 22 were isolated as *Klebsiella spp.* and 10 were isolated as *Staphylococcus aureus*. From antibiotic sensitivity study, Amoxacillin, Cephalothin and Cefotaxim showed 100% resistance whereas Ciprofloxacin, Gentamycin and Nalidixic acid showed 100% sensitive to these isolated microbes.

KEYWORDS: Secondary Infection, Pulmonary Tuberculosis, Microorganisms

INTRODUCTION

Tuberculosis is a common and in many cases lethal infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*. One third of the world's population is thought to have been infected with *M. tuberculosis*, with new infections occurring at a rate of about one per second. In 2007, there were an estimated 13.7 million chronic active cases globally, while in 2010, there were an estimated 8.8 million new cases and 1.5 million associated deaths, mostly occurring in developing countries. The absolute number of tuberculosis cases has been decreasing since 2006, and new cases have decreased since 2002 (Schiffman 2009). The high lipid content of this pathogen accounts for many of its unique clinical characteristics. In nature, the bacterium can grow only within the cells of a host organism, but *M. tuberculosis* can be cultured in the laboratory (Lawn and Zumla 2011). Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air. Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected (WHO 2011). People in the developing world contract tuberculosis because of compromised immunity, largely due to high rates of HIV infection and the corresponding development of AIDS (WHO 2009). Diagnosis of active TB relies on radiology (commonly chest X-rays), as well as microscopic examination and microbiological culture of body fluids. Mixed infection is the simultaneous invasion of more than one species of pathogenic bacteria through the same portal of entry. On the other hand, entrance of infectious microorganism at intervals following the impletion of the primary species is spoken of as "secondary infection". There are several pathogenic species which can survive in lungs beside of normal flora of respiratory tract and may produce lesions with tuberculosis (Southwick 2007). Various pathogenic bacteria and fungi have been found in tuberculous sputum e.g. Streptococci, Staphylococci, Pneumococci, *B. Infuenzae, Moraxella catarrlis, K. pneumonae*, *P. aureginosa*, Actinomyces and Diptheria like bacilli (Kumar *et al.* 2007). About 90% of those infection with *M. tuberculosis* are asymptomatic (latent TB infections, sometimes called LTBI); only a 10% has lifetime chance

which will progress to overt, active tuberculous disease. In those with HIV, the risk of developing active TB increases to nearly 10% a year. If effective treatment is not given, the death rate for active TB cases is up to 66% (Niederweis *et al.* 2010).

TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages. The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe or the lower part of the upper lobe. Tuberculosis of the lungs may also occur via infection from the blood stream. This is known as a Simon focus and is typically found in the top of the lung. This hematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain and the bones. All parts of the body can be affected by the disease, though for unknown reasons it rarely affects the heart, skeletal muscles, pancreas or thyroid (Michael and Peter 2005). Tuberculosis is classified as one of the granulomatous inflammatory diseases. Macrophages, T lymphocytes, B lymphocytes and fibroblasts are among the cells that aggregate to form granulomas with lymphocytes surrounding the infected macrophages. The granuloma prevents dissemination of the mycobacteria and provides a local environment for interaction of cells of the immune system. Bacteria inside the granuloma can become dormant, resulting in latent infection. Another feature of the granulomas is the development of abnormal cell death (necrosis) in the center of tubercles. To the naked eye, this has the texture of soft, white cheese and is termed caseous necrosis (Golden and Vikram 2005). If TB bacteria gain entry to the bloodstream from an area of damaged tissue, they can spread throughout the body and set up many foci of infection, all appearing as tiny, white tubercles in the tissues. This severe form of TB disease, most common in young children and those with HIV, is called miliary tuberculosis. People with this disseminated TB have a high fatality rate even with treatment (about 30%) (Houben *et al* 2006). Tissue destruction and necrosis are often balanced by healing and fibrosis. Affected tissue is replaced by scarring and cavities filled with caseous necrotic material. During active disease, some of these cavities are joined to the air passages bronchi and this material can be coughed up. It contains living bacteria, and so can spread the infection. Treatment with appropriate antibiotics kills bacteria and allows healing to take place. Upon cure, affected areas are eventually replaced by scar tissue (Herrmann and Lagrange 2005).

Diagnosis of latent TB relies on the tuberculin skin test (TST) and/or blood tests. Treatment is difficult and requires administration of multiple antibiotics over a long period of time. Antibiotic resistance is a growing problem in multiple drug-resistant tuberculosis (MDR-TB) infections. Prevention relies on screening programs and vaccination with the bacillus Calmette–Guérin (BCG) vaccine (Schiffman 2009). Multi-drug-resistant tuberculosis (MDR TB) occurs when TB patients stop taking their prescribed medications or do not take them as directed. Patients often stop taking the drugs when they begin to feel better or to avoid side effects. However, TB bacteria can survive inside the body for several months during treatment and are ready to spring back into activity when the medication disappears (Maryn 2007). Symptoms return with a vengeance, and infected people become highly contagious again, putting those close to them at risk. In MDR TB, germs become stronger than the antibiotics, making the drugs less effective. Patients with MDR TB need special medications, but they may not work as well. In addition, patients can spread this highly dangerous form of the disease to others (Lawn and Nicol 2011). One way to fight this problem is through Directly Observed Therapy (DOT). In DOT, patients must take their medications regularly in the presence of a health professional. Home visits by health professionals to supervise the taking of medications or free transportation and meals often are provided to encourage patients to take part in this type of program (O'Brien 1994). A TB vaccine is given to infants and toddlers in countries with high levels of the disease. The vaccine is not commonly used in the United States because it does not always work and it may cause a positive skin test, making it more difficult to detect true TB infection. In present study, attempt was taken to

determine the secondary infection in lungs of pulmonary TB (PTB) patients, their antibiotic sensitivity pattern and statistical analysis of new and follow-up PTB patients.

MATERIALS AND METHODS

TB suspected patients were sent to the pathology department of upazilla health complex, Tongibari, Munshigonj, Bangladesh by Government physician, Private practitioner, Village doctor, NGO [BRAC] and other field staff. All of them were suffering from more then three weeks cough, mild fever and chest pain and some of them were weight loosing. Three subsequent days cough were collected from patients. Totally 450 patients were examined their cough under these study. Several culture media were used in this study (reagent grade) which includes MacConkey's Agar, Blood Agar, Nutrient Agar, Simmon Citrate Agar and Motility Indole Urease. Sputum sample were inoculated directly onto MacConkey's agar, Blood agar and Nutrient agar plates. The plates were then incubated overnight at 37^{o}C and examined for various bacterial colonies. Samples were collected in special room with high ventilation system. Patients were informed that the samples were not saliva, but from the deep respiratory system, and the patient were washed their mouth well to remove the food particles. The sample volume was between (2-5 ml) and those were contain purulent and mucous and not saliva only. The external surface of the containers was clean. The patient washed their hands with water soap after collecting the sample. The smear was stained by ziehl-neelson stain method to identified the tubercular bacilli under microscope (Anthony 2005).

RESULTS AND DISCUSSIONS

Detection of PTB Patients Sputum sample was taken from male and female patients and directly performed AFB stain procedure for isolating of active pulmonary tuberculosis patients. Total 450 patients were examined sputum as TB suspect patients under this study. Among the total TB suspected patients, 44 patients were identified as smear positive Tuberculosis Patient. But in later, more eight patients were identified as TB patients by Chest X-ray, MT test and other pathological evidence who were smear negative cases. It was observed that among the total patients, 261 were male and 189 were female patients. 44 persons were detected as smear positive pulmonary tuberculosis patient that means *Mycobacterium tuberculosis* had found directly under oil immersion microscopy. On the other hand, 08 patients were also detected as smear negative (Table 1).

PTB Patients in New and Follow–Up Cases During this study, 100 samples [sputum] were cultured for identification of secondary bacteria in both sex and various age grouped patients. According to age, all patients were divided into four categories. There was no patient in 0-15 yrs. group, but patient number in 16-45 yrs. group in both sex more high than other groups. Samples were collected from new diagnosis and follow-up patients whose were already treated by TB drugs. 52% samples were collected from new patients who were not treated by TB drugs yet.

Biochemical Test Analysis Different types of bacterial strains were isolated through appropriate biochemical test. *Staphylococcus* spp. showed the positive coagulase and catalase result whereas *Klebsiella* spp. showed positive in lactose fermenting reaction, urase and citrate utilization. On the other hand, *Psuedomonas* spp. showed oxidase positive result.

The Proportion of Secondary Infection in all Patients Categories In this study, 100 persons were taken under the study where patient were categorized into five different groups- new smear positive case, new smear negative case, 2nd month follow-up case, 5th month follow-up case and 6th month follow-up case during TB treatment. The secondary infection was much higher in new cases patients. A few number of secondary bacterial infection had found in lungs of PTB patients whose were treated already TB drugs but this infection was slightly increasing in last part of the TB treatment. A large number of *Klebsiella* spp. (22) was isolated as secondary bacteria in lungs from PTB patients and a significant

number of *Staphylococcus aureus* (10) also isolated. A few number of *S. pyogen* (3), *M. cattaralis* (1) and *P. aeroginosa* (2) were found in this study. It was observed that male (58%) patients were higher than female (42%) and youth male were more infected in PTB and secondary infection.

Antibiotic Susceptibility Test There are 8 antibiotic discs were used for antimicrobial sensitivity. But most of those antibiotic were resistance against isolated bacteria except Ciprofloxacin, Gentamycin and Nalidaxic acid but in some antibiotics were showed moderate sensitivity like; Tetracyclin, Streptomycin. Amoxacillin, Cefotaxme, Cephalothin were showed total resistant (Table 4). Ciprofloxacin, Gentamycin, Nalidixic acid were showed 100% sensitivity along all bacterial strains. in the contrast,Tetracyclin and streptomycin were showed moderate sensitivity to *S. aureus , S. pyogen* and *K. pneumonae*. Otherwise Amoxacillin, Cephalothin and Cefotaxim were showed total resistant in all bacterial strains. Ciprofloxacin, Gentamycin and Nalidixic acid showed 100% sensitivity whereas Amoxacillin, Cephalothin and Cefotaxime were showed 100% resistant. In previous study (Arch and Mainous 2010), 50 PTB Patients were taken under that study where two catagories were included - new case and failure case after treatment. 72% patients were infected with secondary infection.

REFERENCES

1. Anthony H (2005) TB/HIV a Clinical Manual. (2nd ed.). Geneva: World Health Organization. pp. 75. ISBN 978-92-4-154634-8.
2. Arch G and Mainous III (2010) Management of Antimicrobials in Infectious Diseases: Impact of Antibiotic Resistance. Humana Pr. pp. 69. ISBN 1-60327-238-0.
3. Golden MP and Vikram HR (2005) Extrapulmonary tuberculosis: an overview. American Family Physician, 72 (9), 1761–8.
4. Houben E, Nguyen L and Pieters J (2006) Interaction of pathogenic mycobacteria with the host immune system. Curr Opin Microbiol ., 9 (1), 76–85.
5. Herrmann J and Lagrange P (2005) Dendritic cells and *Mycobacterium;* Jindal, SK. editor-in-chief, Textbook of pulmonary and critical care medicine. New Delhi: Jaypee Brothers Medical Publishers. pp. 549. ISBN 978-93-5025-073-0.
6. Kumar V, Abbas AK, Fausto N and Mitchell RN (2007) Robbins Basic Pathology (8th ed.). Saunders Elsevier. pp.516–522. ISBN 978-1-4160-2973-1.
7. Lawn, SD and Zumla, AI (2011) Tuberculosis. Lancet, 378 (9785), 57–72.
8. Lawn, SD and Nicol, MP (2011) Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future microbiology, 6(9), 1067–82.
9. Maryn M (2007) Totally Resistant TB: Earliest Cases in Italy. Euro Surveill. 12(20): 3194.
10. Niederweis M, Danilchanka O, Huff J, Hoffmann C and Engelhardt H (2010) Mycobacterial outer membranes: in search of proteins. Trends in Microbiology, 18 (3), 109–16.
11. O'Brien R (1994) Drug-resistant tuberculosis: etiology, management and prevention. Semin Respir Infect 9 (2), 104–112.
12. Michael A and Peter GG (2005) Evidence-based respiratory medicine. Oxford, Blackwell. pp. 321. ISBN 978-0-

7279-1605-1

13. Schiffman G (2009) Tuberculosis Symptoms. eMedicine Health. www.
14. Southwick F (2007) Chapter 4- Pulmonary Infections. Infectious Diseases: A Clinical Short Course, 2nd ed. McGraw-Hill Medical Publishing Division. p.104. ISBN 0-07-147722-5.
15. World Health Organization (2009) Epidemiology. *Global tuberculosis control: epidemiology, strategy, financing*. pp. 6–33. ISBN 978-92-4-156380-2.
16. World Health Organization (2011) Global tuberculosis control–surveillance, planning, financing. pp. 41–43. ISBN 978-92-4-156380-2.

APPENDICES

Table 1: Detection of Active PTB Patients by AFB Stain in Both Sex

Sex Group	Smear Positive PTB	Smear Negative PTB	Smear[-ve] Patients
Male =261	34	03	210
Female=189	10	05	188

Table 2: Patients Were Selected for Sputum Culture in Different Age, Sex and New and Patients under Treatment

Age Group	Sex Group	New PTB Patients		2nd Month Follow-Up Patient	5th Month Follow-Up Patient	6th Month Follow-Up Patient
		Smear [+ve] PTB	Smear [-ve] PTB			
0-15 yrs.	M	-	-	-	-	-
	F	-	-	-	-	-
16-45 yrs.	M	18	2	8	4	5
	F	6	3	4	1	2
46-60 yrs.	M	10	1	5	3	2
	F	3	1	2	2	3
>60 yrs.	M	6	-	3	1	-
	F	1	1	2	-	1

Table 3: Biochemical Test of Isolated Organisms

Organism	Catalase	Coagulase	Oxidase	Lactose-Fermentation	Citrate Utilization	Urase
S. aureus	+	+	-	-	-	-
S. pyogen	-	-	-	-	-	-
K.pneumonae	-	-	-	+	+	+
P. aeruginosa	-	-	+	-	-	-
M. catarralis	+	-	+	-	-	-

+ = positive, - = negative

Table 4: Percentage of Antibiotic Sensitivity Pattern of Isolated Pathogen against Different Commercial Antibiotics

Antibiotics	*S. aureus*		*S. pyogen*		*M. catarralis*		*K. pneumonae*		*P. aureginosa*	
	S	R	S	R	S	R	S	R	S	R
Ciprofloxacin	100%	-	100%	-	100%	-	100%	-	100%	-
Nalidaxic Acid	100%	-	100%	-	100%	-	100%	-	100%	-
Getamycin	100%	-	100%	-	100%	-	100%	-	100%	-
Tetracyclin	-	20%	-	33%	R	R	-	15%	R	R
Streptomycin	-	50%	-	66%	-	-	-	40%	R	R

R= resistant, S= sensitive.

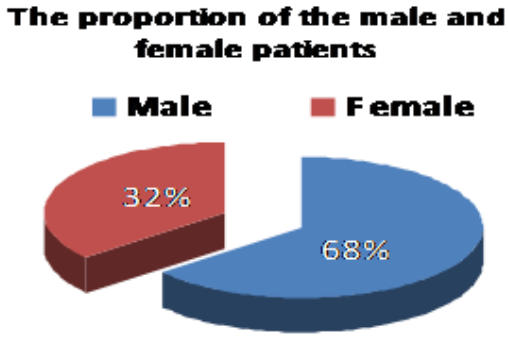

Figure 1: The Percentage between Male and Female Patients Whose Sputum was Cultured for Isolation of Secondary Bacterial Infection

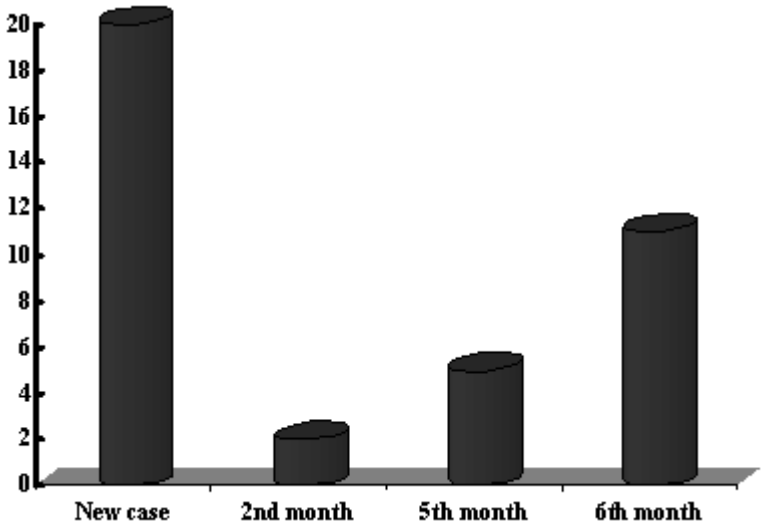

Figure 2: Secondary Infection Rate among Total TB Patients