**Short Communication**

# Comparative analysis for the detection of tuberculosis by conventional and flurosence microscopy

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**Abstract**

Total of 300 TB patients sample were collected from NIDCH (Indoor and Outdoor) for six months period where three different specimens (sputum, pus, BAL) were collected among different age group (male and female). In sputum sample, percentage of identified samples in Auramine-O stain was 41% whereas in Z-N stain 32%; in pus sample, Auramine stain is 22% and Z-N stain 15%; In BAL sample, Auramine stain is 12% and Z-N stain is 10%. Therefore, out of 300 samples, 25% of positive samples were identified by Auramine-O staining whereas 19% of positive samples were identified by Z-N stain. In all the cases Auramine-O is found better for identification of TB than Z-N. In sputum, male positive 10%, female positive 3.66%; in pus sample, male positive 5% and female positive 2.33%; in BAL sample, male positive 3% and female positive 1%. Age group of 26 to 45 years were found high rate of TB in male patients.

**Key Words:** Comparative analysis, TB, Auramine-O and Ziehl-Neelsen staining.

Tuberculosis (TB) is an infectious disease of global impact. According to World Health Organization (WHO) estimates, one third of world's population are harboring TB infection. It was reported that among communicable diseases, TB is the second leading cause of death worldwide, killing nearly 2 million people each year. Most cases are in the under developed countries of the world[1, 2]. TB is a major public health problem in Bangladesh too. In 2007, there were an estimated 353,103 new cases, 1,587,797 of which were sputum smear-positive (SS+) TB cases; more than 70,900 were TB related deaths. In 2008, the WHO ranked Bangladesh sixth among the world's 22 high-burden TB countries. The TB mortality rate (45 death/100,000 populations) in Bangladesh is 45 percent higher than the Southeast Asian region average (31 deaths/100,000 populations).

The estimated prevalence of all forms of TB and incidence rates in Bangladesh was 425 and 225 respectively per 100000/year and the mortality rates was 51/100000/year[3]. The prevalence of tuberculosis is continuing to increase because of the increased number of patient infected with human immunodeficiency virus, bacterial resistance to medications, increased international travel and immigration from countries with high prevalence, and the growing numbers of the homeless and drug abusers[4]. Diagnosis of tuberculosis in developing countries mainly depends on result of stained sputum smear. Treatment regimens are usually prescribed without assessing the drug susceptibility profile of infecting strain[5].

The present study was designed to identify TB patients by Ziehl-Neelsen (ZN) and Auramine-O (LED) staining and compare these results. The study was carried out with the samples of fresh specimens of Sputum, Pus and Broncho-Alveolar Larva (BAL) of total 300 patients (out door and indoor) at National Tuberculosis Reference Laboratory (NTRL) of National Instiute of Diseases of the chest and hospital (NIDCH), Mohakhali, Dhaka for six months period from January 2012 to June 2012 where all ages (10 to 70 years) and sexes (male/female) patients of both- (i) who were treated with anti-tubercular drugs but did not respond and (ii) who did not receive any

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anti-tubercular drugs were considered. Among the specimen, 100 were taken from sputum, 100 from pus and 100 from BAL. Two concentrated (digested and decontaminated) smears were prepared from each sample for staining by using both ZN and LED microscopy techniques according to the standard operating protocol[6]. It was found that *M. tuberculosis* in sputum sample were detected 41% in Auramine stain which is bettter than Z-N (32%) staining method; in pus sample, 22 positive cases were found; in BAL, it was 12 positive (Table I & II). Aftab *et al*[1] showed that a higher number of female patients were suspected of tuberculosis infection than men where out of 5 types of 798 specimens received over a period of 5 years, only 46.3% (n=369) were respiratory, whereas the remaining 53.7% (n=429) were non respiratory tract category samples including sputum, pus, lymph node aspirate, urine and endometrial curetting. They examined the specimens for the presence of acid-fast-bacilli (AFB) in ZN smear, among these 3.1% gave a positive ZN stain while 12.3% were positive on culture and only 15.16% of clinical samples belonging to 5 different categories of specimens received from patients of both sexes with a provisional diagnosis of tuberculosis, tested positive for Mycobacterium both by ZN smear and culture on LJ medium. In present it was observed that the highest positive case were found in age group 26-45 years and the lowest positive case were found in the age group 46-75 years and in sex group, highest positive cases were found in the male (Table III). Out of a total of 369 respiratory tract categories, 10.3% sputum samples were positive for AFB on both ZN and culture whereas among the non respiratory tract category, 28.2% pus, 30.95% lymph node aspirates, 15.6% urine, 3.42% endometrial curetting were reported positive[1]. It was observed by that in males, 8.6% were positive on ZN while only 1.96% females gave a positive ZN smear. They stated that rate of detection was highest in lymph node aspirates, followed by pus and sputum and 64 females and 29 males gave a positive culture results (1-2). 798 biological specimens were examined by Aftab *et al.*[1] for the presence of AFB, where 15.16% was found positive and among these, 3.1% were AFB smear positive with ZN stain and 12.03% were AFB positive on culture with LJ medium. In contrast, a group of workers examined 81 samples of lymph node biopsies from clinically suspected cases of lymphadenitis for AFB and reported that rate of positive cases on culture and AFB smear was 13.6% and 28.4% respectively[7]. However according to a study bacteriological and/or histological confirmation of tuberculosis was obtained in 88% of the case[8]. Whereas Aftab *et al.*[1] showed that a higher number of female patients were suspected of tuberculosis infection than men and in males, 8.6% were positive on ZN while only 1.96% females gave a positive ZN smear. Moreover, in contrast 64 females and 29 males gave a positive culture results[1]. A major number of the sputum specimens, (3.8%), were of females with a provisional diagnosis of pulmonary tuberculosis who remain at increased risk of developing active tuberculosis being disadvantaged and marginalised population. In Aftab *et al.*[1] study it was found that the rate of infection is more in men than in women[9] On the other hand, a recent study also showed that men and women were equally affected[10]. Another study found that the incidence among women peaks at 25–34 years of age. In this age group, rates among

**Table 1: Precentage of positive M. tuberculosis in Sputum, pus and BAL samples**

| Types of sample | Age group | Sex group | | Staining method | | Total positive (%0 | |
|---|---|---|---|---|---|---|---|
| | | Male | Female | Auramine-O | ZN | Auramine-O | ZN |
| Sputum | 10-25 | 7 | 2 | 9 | 7 | 13.66 | 10.66 |
| | 26-45 | 21 | 9 | 30 | 23 | | |
| | 46-70 | 2 | - | 2 | 2 | | |
| Pus | 10-25 | 2 | 1 | 3 | 2 | 07.33 | 05.00 |
| | 26-45 | 12 | 6 | 18 | 12 | | |
| | 46-70 | 1 | - | 1 | 1 | | |
| BAL | 10-25 | 2 | 1 | 3 | 2 | 04.00 | 03.33 |
| | 26-45 | 6 | 2 | 8 | 7 | | |
| | 46-70 | 1 | - | 1 | 1 | | |

**Table 2: Compairative analysis of total number of sample in Auramine-O and ZN staining**

| Sex | Total Number | Total Number of Positives | |
|---|---|---|---|
| | | Auramine-O | ZN staining |
| Male | 170 | 52 | 41 |
| Female | 130 | 23 | 16 |

**Table 3: Compairative study of total sample in different age group**

| Types of sample | Sex group (%) | | Staining method | |
|---|---|---|---|---|
| | Male | Female | Auramine-O | Z-N |
| Sputum | 10.00 | 3.66 | 41 | 32 |
| Pus | 5.00 | 2.33 | 22 | 15 |
| BAL | 3.00 | 1.00 | 12 | 10 |

women are usually higher than those among men[11]. Aftab *et al.*[1] indicated that in the diagnosis of TB, Auramine-O has greater sensitivity than ZN; specially, in case of a single specimen, the diagnostic value of Auramine-O is quite significant. In this study, Auramine-O staining method was found more sensitive than the ZN staining method.

In spite of progress made in promoting public health, the increase in tuberculosis has taken communities in many countries by surprise indifferent regions of the world. Even in the twenty first century, this old disease countries to be a problem. Each year, approximately 2 million persons worldwide die of tuberculosis and 9 million become infected. After contamination *M. tuberculosis* multiplies slowly, in most cases in the terminal alveoli of the lung and in the lymph node of the corresponding area which represents the primary infection. Post pulmonary TB may occur after months or year without clinical signs due to reactivation of dormant bacilli may be in response. Re-infection of a person who had a previous primary infection may also lead to active TB. The risk of development an active TB depends on host immune defense and bacterial load. Since the introduction of anti-TB treatment, a rapid reduction of annual risk of infection has been observed in many industrialized countries, with the infection risk diminishing by approximately 50% every 5 to 7 years during this period. This tendency was observed in countries having a BCG vaccination program as well as in those without one. From an epidemiological point of view, the BCG vaccination is therefore justified by its direct effect, but it is not a good tool to reduce transmission. Direct Staining for Acid fast bacilli (AFB) is the most rapid method, and takes less than 1 hour. However, for sensitivity, microscopic examination requires a large number of bacteria ($>10^4$/ml) in clinical sample. The limit of detection with this method is that it requires at least 5x10 bacilli/ml of sputum. Moreover, it can not distinguish *M. tuberculosis* from other Mycobacteria and considered useful only as a screening test[12]. Fluorescence microscopy with fluorochrome dyes such as auramine-0 or auramine-rhodamine is ruputed to have higher degrees of sensitivity and specificity and is thus a more accurate test for the diagnosis of TB. However, it is mainly performed in developed countries because it is more expensive than conventional method, as it requires unstable expensive fluorescent staining reagents, which increases the cost of the assay, thus hindering its widespread use in developing countries[13]. There are several methods of determining the acid-fast nature of mycobacteria. Smear Microscopy in the simplest and most rapid procedure currently available to detect AFB in clinical specimen[14]. In many countries Diagnosis of TB is performed by microscopic examination of a stained sputum smear by Z-N staining. The main advantage is, it is inexpensive, simple, easy to perform and specific. The advantage of fluorescence microscopy is that a low magnification objective is used to scan smear, allowing a much larger area of the smear to be seen and resulting in more rapid examination. On drawback is using a low magnification is the greater probability that artifacts may be mistaken for acid-fast bacilli. It is therefore strongly recommended that suspect bacilli be confirmed at a higher magnification[15]. The use of Auramine-O as a fluorescent method to deect Micobacteria in sputum was proposed many years ago and re-evaluated later using a combination of auramine O and rhodamine[16]. It is therefore, usually accepted that the fluorescent method should be given performance over the Z-N and kinyoun method. In general the fluorescent method should be used by laboratories with larger specimen number. It is more expensive then the ZN staining requiring a fluorescent microscope[17]. Several studies have been performed to asses the usefulness of adding a chemical reagent, sodium hypochorite, to liquefy and then concentrate the sputum by further centrifugation to increase sensitivity. However, for several reasons, sodium hypochtorite also known as bleach method is not routinely used in many setting[4]. It is, therefore, possible to conclude that both ZN and Auramine-O can be used for the diagnosis of TB, however,

if only one or two specimens are available, Auramine-O is preferable. The ZN method has commonly been used around the world, particularly in developing countries, because of its simplicity and low cost[18]. One disadvantage of the technique is that it may sometimes yield false positive results. However, most of these can be prevented by restaining the smear[19]. Aftab *et al.*[1] showed that the rate of detection of Mycobacterium was highest in lymph node and pus than sputum. In contrast some other studies found that the sputum is the major infectious sample, followed by biological liquids (9, 20) where it was observed that ZN smear examination had a sensitivity of 33.79% and a specificity of 100%. For LJ media, sensitivity was 48.9% and specificity was 100%[21]. A group of workers also found that the ZN stain is the primary procedure for detection of Mycobacterium[12]. However, other studies found that besides bacteriology, histopathology is a complimentary diagnostic tool for detection of TB granuloma in tissues[8] where they suggested that in TB epidemic areas, most of the cases of TB can be diagnosed correctly by simple and cheap methods which are generally available at district hospital level. Although acid fast bacilli (AFB) microscopy, and conventional Lowenstein Jensen (LJ) culture remain the cornerstone of the diagnosis of TB, these traditional bacteriological methods are either slow or their sensitivity is quite low, especially with clinical samples that contain small number of organisms. This can affect treatment by either delaying it or causing inappropriate empiric therapy for TB to subjects without Mycobacterial infections or with Atypical Mycobacteria[21]. There is an urgent need to promote the use of Bactec for early detection and drug susceptibility and real time PCR for even more rapid diagnosis in developing country like us[22].

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