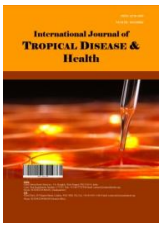

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Value Addition on Trend of Tuberculosis Disease in India- The Current Update

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Authors' contributions

This work was carried out in collaboration with all authors. Author PKG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MHN and SSM managed the analyses of the study. Authors SC, AS and RSS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Tuberculosis (TB) is infectious diseases were the lungs are mostly affected. It is caused by the *Mycobacterium tuberculosis* bacteria and is spread when a person already affected with TB coughs, sneezes, spits, laughs, or talk. Even though it's is contagious does not easily catch i.e. chances of catching TB are much higher with someone you live with or work than from a stranger. Multidrug-Resistant TB (MDR-TB) arises when the antibiotic fails to kill bacteria. MDR-TB can be treatable and curable with specific anti-TB drugs but unfortunately, these are limited in quantities or not readily available. As per WHO around 4,50,000 people developed MDR-TB in the year 2012. People with a weak immune system are at maximum risk of active TB development. For instance, HIV conquers the immune system, making it harder for the body to control TB bacteria. People infected with both HIV and TB are 20-30% more probable to develop active TB than those who do not have HIV. Besides, WHO estimates, every year 9 million people get sick with TB and 3 million

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with these are “missed” by health systems. Among the top 3 causes of death in women between 15- 44 TB is one the major cause. The symptoms of TB may be mild for many months and can infect 10-15 other people through close contact. This study involves a comparative evaluation of the presence of Tuberculosis concerning factors such as socioeconomic status, sex ratio, age, addiction of nicotine or alcohol, etc. All the screenings were based upon various methods of diagnosis used in pulmonary tuberculosis such as Ziehl Neelsen staining, culture on L.J media, Petroff’s concentration method, and DNA PCR method.

Keywords: Tuberculosis; Mycobacterium tuberculosis; cause and symptoms; treatment and management; public health; a trend of tuberculosis.

1. INTRODUCTION

Respiratory tract infection may be viral or bacterial. Lung infection by bacterial pathogens usually occurs via direct inoculation of an organism through aspiration. The bacteria that cause TB are spread when an infected person coughs or sneezes. Most people infected with the bacteria that cause tuberculosis don't have symptoms. When symptoms do occur, they usually include a cough (sometimes blood-tinged), weight loss, night sweats, and fever. Treatment isn't always required for those without symptoms. Patients with active symptoms will require a long course of treatment involving multiple antibiotics. The acute or chronic communicable respiratory tract infection is caused by *Mycobacterium Tuberculosis* which usually involves the lung but may affect any organ or tissue in the body [1,2].

Detection of the granuloma and epithelioid cells with causation is only supportive and provides the clue that there is some granulomatous disease but to establish the etiological diagnosis of tuberculosis demonstration of *Mycobacterium tuberculosis* in smear /culture is required. Mycobacterium are slow-growing bacteria and hence culture is not routinely done in all laboratories and only a few studies have tried to correlate the cytological finding with microbiological results. Tuberculosis detected as far back as 10,000 BC remains a major public health problem worldwide *M. tuberculosis* infects a third of the world population. Seventy-five percent of tuberculosis patient cases in developing countries are in the economically productive age group (15-50 yrs.) [3,4]. The disease infects one percent of the world’s population each year. It is ironic that though we possess curative chemotherapy and innovative drug delivery strategies such as directly observed therapy and prevention that could eliminate tuberculosis, yet the forecast figures tell otherwise [2].

2. METHOD OF DIAGNOSIS

Although preparation of smear and staining by the Z-N technique is easy to perform. But the AFB positivity is very rare in lymph node aspirates [5]. Methods for diagnosis of tuberculosis have improved in recent years and several molecular techniques for its diagnosis have been introduced for clinical use. Several methods for DNA / RNA amplification are sensitive and specific for the rapid detection of mycobacteria [6]. These methods provide several advantages over culture including confirmation of the presence of mycobacteria within 1-3 days as compared with 2 to 6 weeks with culture techniques. In this regard, polymerase chain reaction amplification of mycobacteria tuberculosis can provide much-needed help. The present study was designed to evaluate the sensitivity, Z-N smear, culture, and DNA PCR in the diagnosis of pulmonary tuberculosis [6,7].

2.1 Strains of *Mycobacterium*

Five strains of *Mycobacterium* have been identified: human, bovine, avian, murine, and piscine, but only the human and bovine strains are pathogenic to man. Thus, the disease is perpetuated throughout the world by man to man transmission and the drinking of infected milk. Improved living standard public health measures and more effective therapy have brought about a remarkable decline in the prevalence of this disease in economically developed nations [8].

Although the control has been satisfactory in many parts of the world, it persists as a major clinical problem. Even in economically developed countries the crowded slums of virtually every urban center, as well as the economically deprived rural area, continue as high -incidence pockets of disease [9]. Occupational hazards account for a markedly increased prevalence of tuberculosis among physicians, hospital personnel, and laboratory workers. Silicosis,

diabetes mellitus, congenital heart disease, and in fact, any chronic deliberating illness greatly predispose to tuberculosis. In many regions of the world where pasteurization of milk is not routine, bovine tuberculosis is still rampant. Fortunately, man has a relatively high natural resistance to M. tuberculosis. As a consequence, on the first exposure, the organism may gain a portal of entry cause relatively trivial lesions but never produce significant disease [10]. Probably no more than 5 percent of Americans now infected with *M. tuberculosis* will ever develop clinical disease. Thus, with tuberculosis, we must distinguish between infection and disease. In older individuals, the disease usually represents the activation of latent infection acquired during childhood. It is estimated that about 75 percent of symptomatic cases in the United State represent activation of such latent childhood infection [11].

2.2 The Agent *Mycobacterium tuberculosis*

Mycobacterium belongs to the family mycobactericidal of order actinomycetal. They are aerobic, non-spore-forming, non-motile, bacillus with high cell wall content of lipid. The growth is slow, the generation time is 15- 20 hrs. Due to its typical cell wall with high molecular weight lipids. Mycobacterium is acid-fast, which means it resists decolorization with acid and alcohol. The following mycobacteria have been isolated [11,12]:

- Lepra bacilli by (*M. leprae*) by Hensen (1874)
- Mammalian Tubercle bacilli (*M. Tuberculosis*) by Koch (1882)
- Bovine Tubercle bacilli (*M. bovis*) by Smith (1900)
- Avian Tubercle bacilli (*M. avium*) (1889) quoted by Rao (1972)
- Johne's bacillus (enteritis in cattle) John of Frothigham (1895)
- *Saprophytic bacilli*
- *M. butyricum* (Butter bacilli)
- *M. pheli* (Timothy gross bacillus)
- *M. stereosis* (Mist bacillus)
- *M. smegmatis* (Smegma bacilli)

During the year 1885 to 1906 the number of workers demonstrated the existence of saprophyte acid-fast bacilli, a group does not appear capable of causing a progressive disease. In 1937, Wells isolated an acid-fast bacillus from voles (microtegrestis) suffering from natural tuberculosis. This organism differs in certain respects from the other mammalian types and is best referred to as the murine type tubercle bacillus as *Mytuberculosis varmuris* [13].

2.3 Pathogenesis

Mycobacterium tuberculosis generally enters the body via the respiratory tract and undergoes hematogenous and lymphatic dissemination. Droplets nuclei of 5 µm or less in diameter are generated by an individual with TB (by speaking, laughing, or sneezing) and are subsequently inhaled by a susceptible, non- immunized person. The number of bacilli in a 5 µm droplets nucleus is estimated to be 1-10. A single organism has the potential to cause the disease, but usually, 5-200 bacilli are necessary for human infection. Inhaled bacilli become lodged in a distal respiratory tract or alveolus, subpleural in location. Once deposited in the alveolar space, bacilli are ingested by non-activated alveolar macrophages at the site of implantation and are subsequently transported to the hilar and mediastinal nodes. Hilar and mediastinal lymph nodes are the first lymphoid tissues organ encountered in the lymphatic spread from the lung parenchyma [14]. This involvement may occur at the time of primary infection or may occur later in life due to reactivation of the previous infection. Tonsils are also an important portion of the entry. The infection may then spread via the lymphatics to the nearest cervical lymph nodes. In the initial stage, the nodes may be discrete clinically. Per adenitis results in matting and fixity of lymph nodes. The lymph nodes coalesce and break down to form caseous pus. This may perforate the deep fascia and present as a fluctuant swelling on the surface (collar-stud abscess), the overlying skin becomes indurated, breaks down, and leads to the formation of a sinus which if untreated may remain for years. Healing may occur with each of three stages with calcification or scarring [14,15].

2.4 Formation of Garnuloma

Activated macrophages and lymphokine-activated T. lymphocytes have enhanced the capability to destroy intracellular bacilli. The resulting inflammatory process is pathologically recognized as a tubercle or granuloma, while it is a collection of modified histiocytes surrounded by lymphocytes and capillary. The granuloma has central necrosis with caseation that inhibits both macrophages function and bacillary growth and results in a loss of cellular structure. Radiologically the resulting lesions are referred

to as a "Ghon's focus ". Tubercle bacilli – free or within macrophages drain along with regional per bronchial lymphatics channel to tracheobronchial lymph nodes which results in caseating granuloma within the hila [12].

2.5 Morphology, Staining and Microscopic Evaluation of Sputum

The *acid-fast bacilli* are slender, straight, and slightly curved rod-shaped organisms, with rounded pointed or sometimes expanded ends. In the tissue, they may occur singly or in pairs often forming an obtuse angle or in a small bundle of parallel bacilli. In the animal body, they are generally larger and thinner than culture. The usual size is 2.3-3.5 µm by 0.3-0.5 µm. The organism is non-motile and non-spore-forming, through it possess considerable powers of resistance to drying. Repeated sputum examination is important because of intermittent excretion of tubercle bacilli by the patient. It was found that the direct examination of sputum specimens from 611 patients was positive in 51.5% of the cases on the first examination, 14.2% more on second examination [16-18]. American Lung Association (1974); Takahashi (1975); David HL (1976) and International Union against Tuberculosis (1977) have reported various cases of false positive or false negative reports [19].

A comparative study for AFB staining on fine-needle aspirates from lymph nodes and other sites in suspected cases of tuberculosis, between ZNS and fluorescent staining methods, were done. Overall AFB positivity by ZNS was 33.5% and fluorescent staining 45.4% when the two methods were combined AFB positivity was 58.7%. Fluorescent staining was superior to ZNS in the presence of a low bacterial load as seen in smear with diagnostic morphological features of TB when the bacterial load is high ZNS is nearly as good as the fluorescent method. Several studies are also conducted to evaluate the relative merits of microscopy and culture [20].

3. MATERIALS AND METHODS

3.1 Selection of Cases

Patients In the study are attending the OPD of T.B and Chest Disease and other departments of S.R.N Hospital and some are also those who have been admitted to T.B and Chest ward of S.R.N hospital, a tertiary health care center, Allahabad. Those patients who have not taken any sort of anti-tubercular treatment or if taken, the total duration of treatment is not more than a fortnight, were included in the study with either of the following inclusion criteria :

- History of cough with expectoration, low-grade evening rise fever, generalized weakness, malaise, hemoptysis.
- Having clear or doubtful radiographic evidence of tuberculosis lesions.
- HIV positive with respiratory symptoms
- Patients with respiratory symptoms with a history of close contact with tuberculosis.

Detailed clinical history of every patient was taken and a thorough physical examination with more emphasis on the respiratory system was done.

3.2 Investigation

The enrolled patients were made to undergo the following investigations:

3.2.1 Skiagrams of chest-PA-view

An X-ray chest view was done in each case. Lateral and other views, if necessary were done to know the size, extent, and nature of lesions. Based on radiological examination, the cases were classified into three groups: according to criteria of the National Tuberculosis Association of the USA [21,22]:

- **Minimal Lesions:** Unilateral or Bilateral lesions with slight to moderate density with total extent equivalent to the volume of lung present above the lower border of the second condro sternal junction and the spine of the fourth or the body of the fifth thoracic vertebra, without evidence of cavitations.
- **Moderately Advanced:** Unilateral or Bilateral lesions, when lesions are slight to moderate density with total extent equivalent to the volume of one lung or dense and confluent lesions limited to the extent to the one-third volume of one lung if cavity present total diameter of all cavities should not exceed more than 4 cm.
- **Far Advanced:** Lesions more extensive than moderately advanced.

3.3 Sputum Examination for Detection of Bacilli

Collection of Sputum: First-morning sample of sputum under adequate aseptic precautions

were taken in a sterilized wide-mouthed universal container with a tightly fitting screw-capped lid. The patients were properly instructed to rinse their mouth before the collection of sputum specimens to avoid contamination with food and other particles and to cough forcibly to bring out the secretion from lower respiratory passages. Patients were advised, not to expose sputum specimens to direct sunlight, excessively hot or radiation.

3.3.1 Laboratory techniques

Collected samples were processed in the following ways after being received in the laboratory.

- **Direct Smear Examination:** A direct smear from thick, purulent cheesy materials in the sputum. The prepared smear was dried in the air and heat-fixed by passing the slide three times over a flame.
- **Petroff's Methods (Concentration Method):** This method was applied to sputum. First, the Equal volume of the specimen and 4% NaOH were mixed in a sterile test tube and vertex mixed and then kept in the incubator at 37°C for 30 minutes. The tube was shaken intermittently. This holding time was decreased to 20 minutes for pleural fluids. The mixture was centrifuged at 3000 rpm for 30 minutes and supernatant fluid poured off. The deposit was neutralized with 8% Hydrochloric acid which was added drop by drop. The reaction of the mixture being tested by adding a drop of phenol red solution to the tube. A specimen was prepared from this deposit, was fixed by passing the slide three times over flame and stained by Ziehl-Neelsen staining as described by International Union against Tuberculosis [23]. Smeared part of each slide was covered with a piece of filter paper and the whole surface of slides was covered with carbol fuchsin solution. Slides were heated very gently until the rise of vapors and kept 5 minutes. Filter papers were removed with forceps and slides were rinsed in a gentle stream of water until all free stain washed away. All slides were covered individually with 25% sulphuric acid for 3 minutes. Slides were rinsed in a gentle stream of running water. Slides were again decolorized with 25% sulphuric acid for 1-3 minutes. Until all colors practically disappeared. Slides were rinsed with water. Decolorized rinsed slides were flooded with 0.3% methylene blue for counterstain and kept for 60 seconds. After rinsing in water, the slides were allowed to dry in air.

All these stained slides were examined under oil immersion lens for AFB. AFB appears as pink or red beaded bacilli against the blue background. Quantitative analysis was done as per the criteria of the International Union Against Tuberculosis [23]. This applied only for the direct method for sputum smear. The minimum 100 oil immersion field has examined. The deposit of the sputum after Petroff's method was inoculated on the Lowenstein Jensen method slope.

3.4 Culture on Lowenstein-Jensen's Medium

Two to three drops of the centrifuged deposits were inoculated on the surface of sloped Lowenstein-Jensen medium in inoculating hood taking all precautions to maintain sterility. All the cultured bottles were incubated at 37°C and the slopes were examined for growth daily for the first four days (For rapid growth mycobacteria, fungi, and contaminant bacteria) and then weekly thereafter [24]. Growth occurs in the form of colonies that were dry, rough, raised with the wrinkled surface, initially creamy, and later becoming yellowish or buff-colored. Appeared after 2 weeks on incubation and some even took 7 weeks of incubation. Smeared were prepared from the colonies and stained by the Ziehl-Neelsen method and observed for Acid Fast bacilli and thus confirmed.

3.5 DNA PCR

Aspirated materials such as pleural aspirates (≈200 µl) were taken in a sterile fresh Eppendorf and 200 µl of T.E (Tris EDTA) buffer was added. The mixture was boiled at 85º-90ºC in a water bath for 10 minutes then immediately frozen at -20ºC for 15 minutes. Therefore 40 µl of lysozyme enzyme was added and vortex mixed. It was incubated at 37ºC for 2 hrs and then 10 µl of proteinase K and 56 µl of 10% SDS was incubated at 56ºC for 2 hrs with continuous shaking. It was then kept at 95ºC for 5 minutes. To inactivate proteinase K.500 µl of chloroform and phenol in the ratio of 1:24 (V/V) was added and centrifuged at 12000 rpm for 5 minutes. Three layers of liquid were visible. The upper layer was very carefully transferred to another sterile Eppendorf without disturbing the middle layer (containing protein). To this upper layer 500

µl of pure chloroform was then added and again centrifuged at 12000 rpm for 2 minutes and then the aqueous layer was taken in another Eppendorf to this 600 µl of 100% ethanol was added and kept at 20ºC overnight next day it was again centrifuged at 10,000 rpm for 15 minutes, the supernatant was decanted and the pellet was suspended in 30 µl of distilled water and kept at -20ºC till PCR was started [25].

For PCR, a master mixture was prepared by mixing 5 µl of 10x PCR buffer, 1 µl of dNTPs, 2 µl each of primers 1 and 2, 0.5 µl of Taq. Polymerase, 40.5 µl of distilled water, and 1 µl of DNA samples for each test in PCR tubes. Tubes were then kept in a thermal cycler with a heated lid which was programmed at 3 phases per cycle at 94ºC for 1 minute (denaturation), 60ºC for 1 minute(annealing), and 72ºC for 1 minute (elongation) for a total of 30 cycles. After the first cycle, a prolonged elongation phase at 72°C for 10 minutes was done. The tubes were then taken out from thermal cycler and PCR products (Amplicons) were examined by agarose gel electrophoresis and stained by ethidium bromide. The amplified products obtained after PCR were loaded, along with DNA molecular weight marker, positive control, and negative control in 1.5% agarose gel which was prepared in Tris Borate EDTA buffer (T&E). The agarose was dissolved in T&E by boiling till it became transparent. After cooling the solution till 60ºC, it was poured in the desired UV transparent molds and wells made with the help of a comb. After solidification of gel, amplified DNA samples were loaded with tracking dye in the ratio of 5:1 (8 µl of PCR product and 2 µl of bromophenol blue dye) in the wells of the gel [25].

Electrophoresis was done at 50 volts for 45 minutes to one hour. The bands of DNA migrated in the gel according to their molecular weight. The gel was put in a staining solution of ethidium bromide (0.5 µg/µl) for 30 minutes to 45 minutes. The gel was visualized on a transilluminator under UV rays. DNA bands were identified based on migration in gel along with standard weight marker and positive control [25].

4. RESULTS AND OBSERVATION

4.1 Tuberculosis

A comparative evaluation of Ziehl-Neelsen staining, concentration method, culture on Lowenstein-Jensen medium, and DNA PCR method in the diagnosis of pulmonary and extrapulmonary tuberculosis patients was carried out. A total of 44 clinically suspected cases of tuberculosis at the age of 14-70 years were studied.

Fig. 1. shows the age and sex distribution of the cases. A maximum number of cases (38.63%) were seen in the age group 21-30 (for both males and females) followed by 31-40 years of age group (27.27%). The minimum number of cases (2.27%) belonged to the age group 41-50 years. In the total number of 44 cases, 32 (72.72) were males and 12 (27.27) were females. There was a distinct predominance of males, with the ratio 3:1 over females. There is an almost equal distribution of rural and urban habitant of patients.

Occupation wise 22.73% each were farmers, housewives, and others, followed by 18.18% labours. Whereas almost 14% were students only. Here others include working people, shopkeepers, and some good habitat people Fig. 2.

Fig. 3 shows that the maximum number of cases 26 (59.09%) were from lower socio-economic status followed by middle-lower 8 (18.18) and in middle-upper 4 (9.09). Whereas upper and lower-upper were equal to 3 (6.82) each. The above classification is based on Kuppuswammy ‘s Socioeconomic Status [26].

Out of 44 cases, 13 (29.54) were smokers and all were males. All females were non-smokers. History of tuberculosis among family members and other contacts were enquired. Out of 44 cases, 8 (18.18%) had a positive family history of tuberculosis.

Observing the among 44 cases, cough with expectoration was found in a maximum number of cases (86.34%) followed by fever (50%) cases. Other symptoms in order of frequency were loss of appetite (22.73%), others (20.45%), chest pain and breathlessness in (18.18%) each, and loss of weight and weakness (9.09%), (6.08) respectively.

Fig. 6 shows that out of 44 cases 34 (77.27.09%) were positive for AFB by direct smear. On quantitative analysis and grading the smear AFB positivity, 7 in 1+, 11 in 2+, and 16 in 3+ were present. On observing the concentration method positivity, it was observed that 9 (60.00%) cases were positive for the concentration method in 15 cases out of the total number of 44 tuberculosis patients.

Fig. 1. Distribution of T.B cases in different age groups among 32 males and 12 female cases

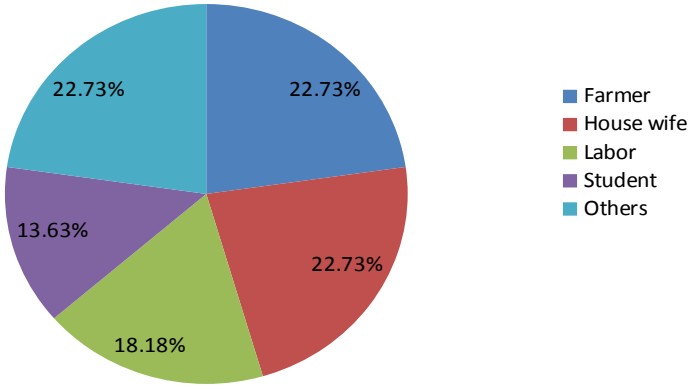

Fig. 2. Distribution of cases among various occupational groups

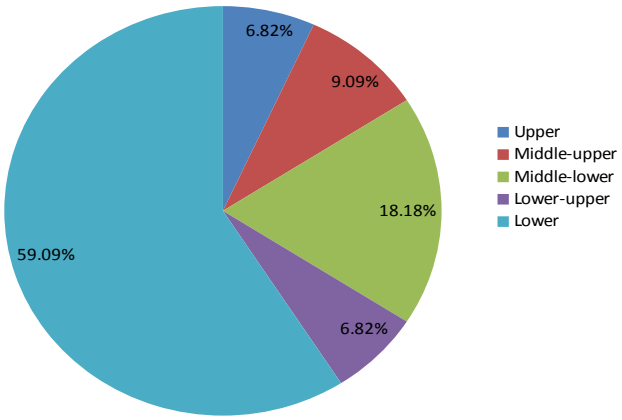

Fig. 3. Distribution of cases among various socio-economic groups

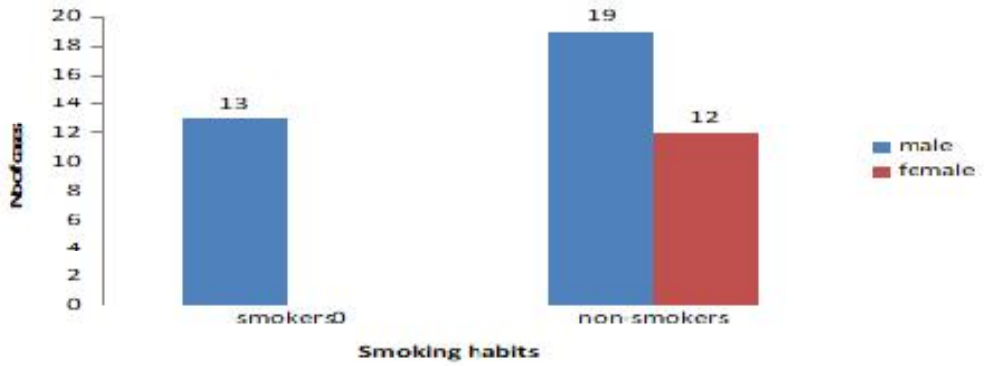

Fig. 4. Distribution of cases according to smoking habits

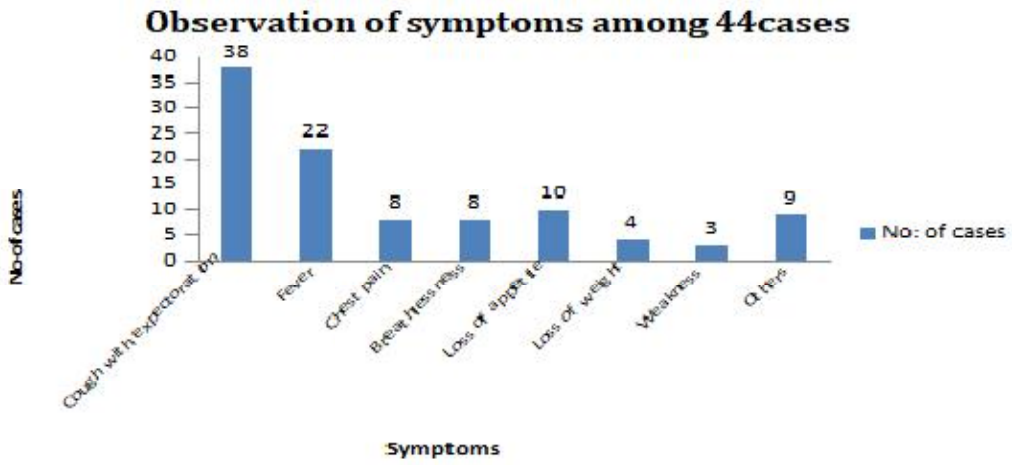

Fig. 5. Graph showing symptoms vs. no of cases they appeared in

On evaluating the culture positivity on the conventional L-J media, it was observed that 7 cases were positive out of 10 patients, whoever gone through culture on L-J media in a total number of 44 tubercular patients.

Fig. 9 shows maximum growth in L-J medium occurred during the 5th week i.e. 2 (28.57%) cases followed by 3rd, 4th, 6th, and 7th with 1 (14.28%) case each. Maximum growth occurred in the L-J medium for tuberculous infected people bacteria during 4th to 6th week with 4 (57.14%).

Fig. 10 shows that DNA PCR positivity was quite effective because it showed 100 affectivities on 2 patients whoever diagnosed through DNA PCR method in the total number of 44 tuberculosis patients.

The graph shows that the first patient was resistant for all first-line antibiotics used in the case of tuberculosis hence he has been categorized under the development of drug-resistant *Mycobacterium tubercle* patient. The second patient showed sensitivity for all antibiotics except *Pyrazinamide*, whereas third showed resistance for both *Ethambutol* and *pyrazinamide* and the fourth patient showed resistance for *Rifampicin* and *pyrazinamide*.

5. DISCUSSION

In the case of chronic pneumonia eg. Tuberculosis, the present study showed a comparative evaluation of Ziehl-Neelsen staining, culture on L-J media, Petroff's concentration method, and the DNA PCR method in the diagnosis of disease. The study also performed an antibiotic sensitivity test. Culture on LJ media was taken as a gold standard and results of the rest of the three methods were analyzed statistically. The present study showed that the

maximum cases were in the age group of 21-30 years (31.63%) followed by 31 to 40 years (27.27%). It can be compared with the observed study of Muddaiah, Ravish Kumar et al 2013, Asmar S et al 2015, and Gholoobi A et al 2014 [27-29].

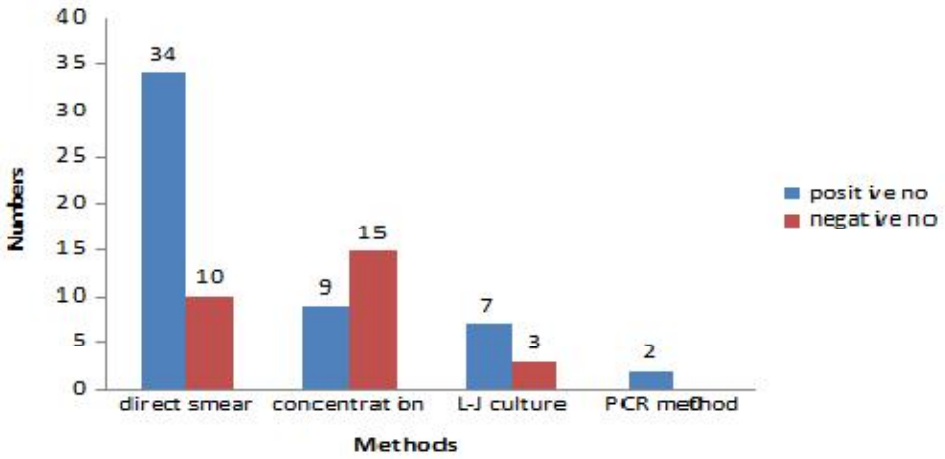

Fig. 6. Comparative evaluation of four methods of laboratory examination

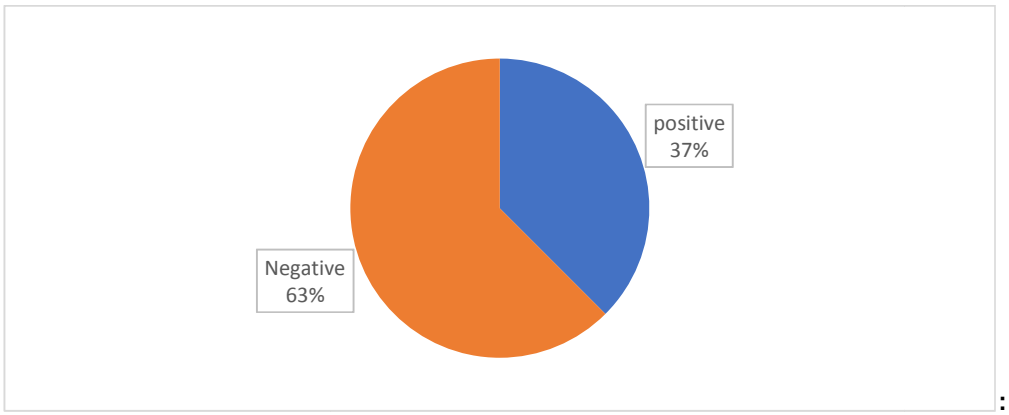

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Fig. 7. Evaluation of AFB positivity by concentration method (out of 24)

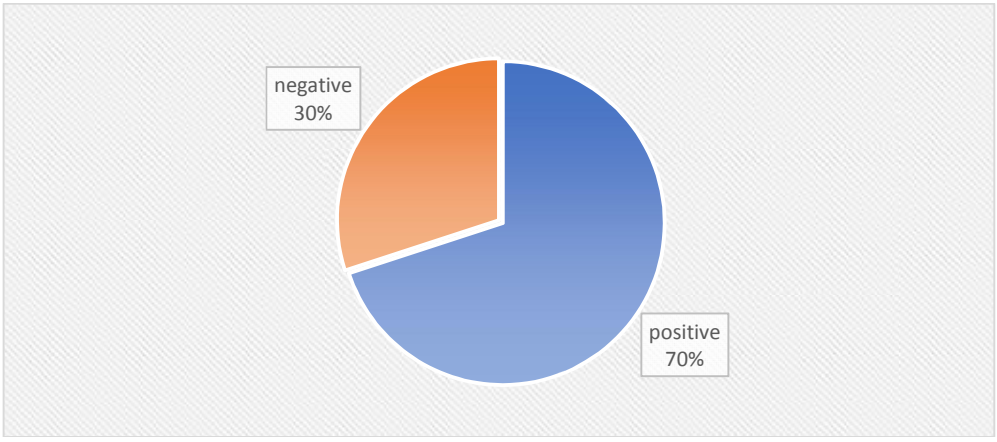

Fig. 8. Evaluation of AFB culture in L-J medium

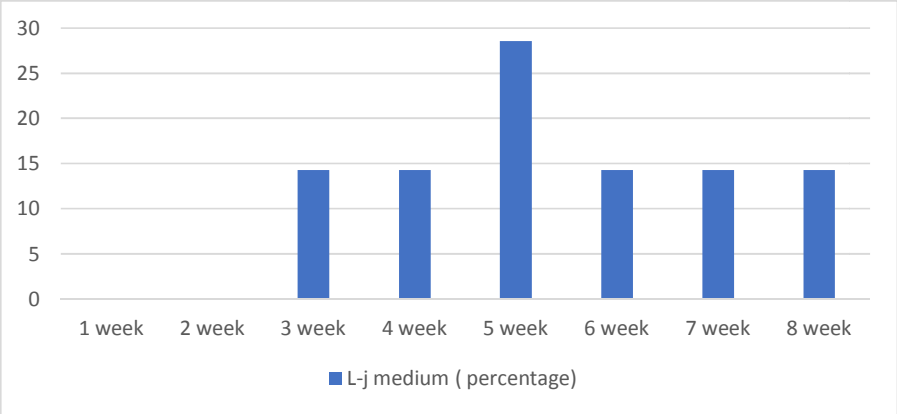

Fig. 9. Evaluation of duration of growth in L-J medium(out of 7 cases)

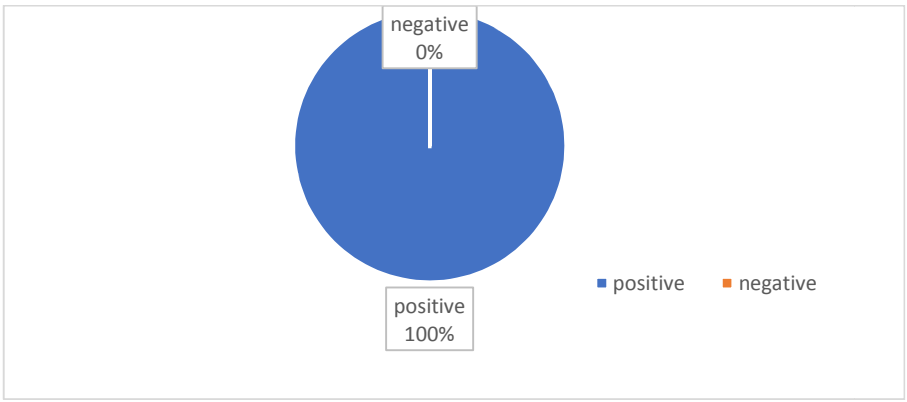

Fig. 10. DNA PCR of fine-needle aspirates for pleural fluid

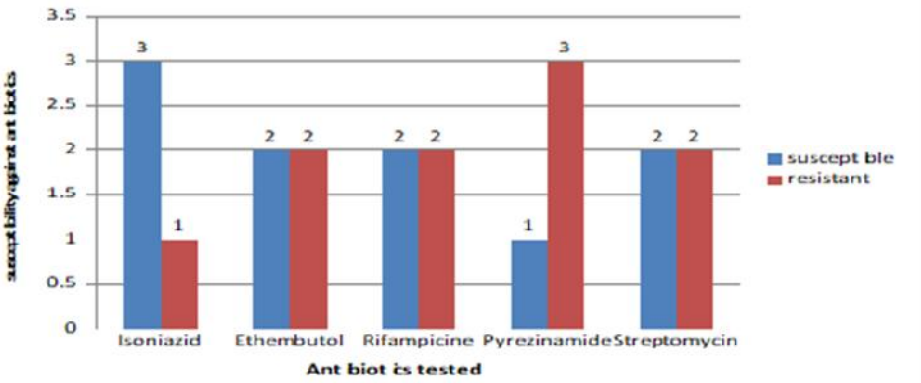

Fig. 11. Antibiotic susceptibility test: The antibiotics susceptibility test was used on 4 patients, out of 7 positive L-J media patients in all 44-observed tuberculosis patient

In the present study males (32) were predominated over females (12 cases) and it can be compared with Horton, Katherine C et al (2016), Rao S (2009) [30,31], however, in some cases, Lau et al (2009) females were dominated [32]. The direct smear is an important diagnostic tool for case finding in Tuberculosis Control Program. It is sensitive, especially in advanced cases, economical and easy to perform and does not require costly equipment. Most of the workers

have advocated its effectiveness while few found it is not so convincing. The present study showed 77.27% positivity. This can be compared with similar findings by Sharma SK et al. [33]. The positivity by direct smear microscopy depends upon criteria for selection of cases and type of sputum collection. Tubercle bacilli are excreted intermittently in sputum and therefore, the yield of a positive result in overnight collection specimens was found more than spot collection specimen irrespective of the method of study on detection of tuberculosis.

All other collected spot specimens in their study except some who also studied overnight collection to compare the spot specimens and showed a very high yield of positivity than our results. This can be explained by the criteria for the selection of cases. In the present study, clinically suspected cases of pulmonary tuberculosis were taken while they selected bacteriological proved or culture-positive cases of tuberculosis for direct smear examination. The results by direct smear examination in our study were similar to the study of Filho & Fonseca (1979) [34]. In the present study, comparing the result of L-J media with direct smear, L-J media showed not higher yield of positivity than direct smear, this can be compared with the observed result found by Naganathan (1979) [35] with a present study which showed positivity of L-J media and direct smear method with 70.00% and 77.27% respectively. Comparing the result of the concentration method with direct smear Naganathan (1979) found the concentration method is not superior to direct smear microscopy because sodium hydroxide has a deleterious effect on the acid fastness of bacilli. The present study showed a similar result with 37.5% positivity of the concentration method 77.27% positivity of the direct smear method.

The positivity and sensitivity of culture also depend on various factors as follows:

- The type of culture media used.
- The load of AFB in the specimen.
- The proper volume of the specimen and its storage.
- Transportation of specimens
- Proper processing of specimens
- The incubation conditions
- Measures are taken to decrease contamination.
- and finally, proper recording of the observations of culture at timely intervals.

The present study showed results of 100% for 2 patients in PCR which can be compared with Popper et al (1994) 96% [36]. For the antibiotic susceptibility test, 4 patients were studied on positive AFB culture in a total number of 44 tuberculosis patients. The present study showed that all first line of tubercular drugs was not effectively sensitive in all 4 patients. This may be due to the evolution of drug-resistant tubercular bacilli, which might be developed form multi-dose of first-line drugs.

6. CONCLUSION AND PROSPECTS

44-patients with acute and chronic lower respiratory tract infection were selected for the study. Sample of the respiratory specimen (Sputum, Bronchial aspirates, Bronchial-alveolar lavage, Pleural fluid, Lung aspirate or fluid, Pus from intercostals drainage, Pus from empyema thoracic) were collected from the patients visiting at Microbiological lab of Motilal Nehru Medical College, Allahabad and a private microbiological clinic. A comparative evaluation of Ziehl-Neelsen staining, concentration method, culture on Lowenstein-Jensen medium, and DNA PCR method in the diagnosis of pulmonary and extrapulmonary tuberculosis patients was carried out. A total of 44 clinically suspected cases of tuberculosis at the age of 14-70 years were studied. The maximum number of cases (38.63%) was seen in the age group 21-30 (for both males and females) followed by 31-40 years of age group (27.27%). The minimum number of cases (2.27%) belonged to the age group 41-50 years. In the total number of 44 cases, 32 (72.72) were males and 12 (27.27) were females. There was a distinct predominance of males with a ratio of 3:1 over females. There is an almost equal distribution of rural and urban habitant of patients. Occupation wise 22.73% each were farmers, housewives, and others, followed by 18.18% labours. Whereas almost 14% were students only. Here others include working people, shopkeepers, and some good habitat people. The maximum number of cases 26 (59.09%) were from lower socio-economic status followed by middle-lower 8 (18.18) and in middle-upper 4 (9.09). Whereas upper and lower-upper were equal to 3 (6.82). History of tuberculosis among family members and other contacts were enquired. Out of 44 cases, 8 (18.18%) had a positive family history of tuberculosis. Observing the among 44 cases, cough with expectoration was found in the maximum number of cases (86.34%) followed by fever (50%) cases. Other symptoms in order of frequency were loss of

appetite (22.73%), others (20.45%), chest pain and breathlessness in (18.18%) each, and loss of weight and weakness (9.09%), (6.08) respectively.

The prevalence of this disease is high among low socioeconomic groups. This can be due to the improper sanitary and unhygienic conditions faced by such groups. Furthermore, antibiotic sensitivity test hints that lack proper and timely medication might have contributed to the fact that many strains were found resistant to, from few to all available antibiotics used for tuberculosis treatment. This might be the consequence of the absence of proper medical staff and medication in rural areas or because of a lack of education about the importance of completion of recommended medication dosage by the medical practitioner. Hence the availability of proper medical staff, proper education, and awareness about the disease, it's symptoms, and prevention can play a key role in lowering the susceptibility of such groups to the disease.

DISCLAIMER

The research publication reported in this manuscript has been done in the Department of Microbiology/ Pathology, MotiLal Nehru Medical College Allahabad, U.P India during the period of 2006-2007. The manuscript was prepared in the Department of Biotechnology, R.V College of Engineering, Bangalore-560059, India, and Department of Life Sciences, Garden City College of Science and Management (Affiliated to Bangalore University), Bangalore-560056 India. We confirm that there is no conflict of interest exists in any of institution mentioned for direct or indirect/known or unknown purpose and therefore, the above-said manuscript publication has no objection from the any of the organization mentioned above. We confirmed that the above-mentioned manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media. All authors listed have contributed sufficiently to the project, and all those who are qualified to be authors are listed in the author by line.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research study was done in the Department of Microbiology, Motilal Nehru Medical College (MNRC) Allahabad (Now known as Prayagraj), U.P India. I would like to acknowledge Dr. Aundita Bhargava, Prof and Head of Microbiology Department MNRC Allahabad for allowing me permission to perform my research during my M. Tech Project course in 2006 and 2007 and to analyse OPD (Outdoor Patients) data during that time. Apart from I would like to mention that this research study was performed to make more people aware of the trends of some deadly diseases such as Tuberculosis hence this detail cannot be used to reproduce any documents of similar kind or to generate any funds or any other benefits. Besides, I would like to mention that the name of patients and their specific locations cannot be revealed to maintain the confidentiality of medical practices.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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