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RESEARCH ARTICLE

A COMPARATIVE MICROSCOPIC & CULTURAL TUBERCULOSIS DIAGNOSTIC STUDY IN RURAL CHENNAI

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ABSTRACT

Background: Care of patients with tuberculosis starts with a quality assured medical treatment. This has several facets like clinical, radiological & laboratory techniques. The laboratory techniques need to be reliable, rapid, cost effective & reproducible not only for the patients but also for the clinicians, as it will lay down the base for early initiation of suitable treatment & reduce the mortality & morbidity of the diseased person.
Objective: To ensure reliable, early detection and cost effective diagnostic technique by comparing two microscopic and cultures.
Design: Case control study, with comparative microscopy & cultural techniques.
Methodology: 75 willing Patients with their consent were selected from the Departments of Medicine &TBCD.25 HCW were taken as controls. The sputum collected were processed for two different staining, microscopic & culture techniques. These procedures were done as per standard protocol. The stained smear were screened & interpreted as per RNTCP guidelines.
Results: Liquid media exhibited growth as early as 10th day, while LJ media had the growth only on the V week. The liquid media half the rate of the solid media.LED microscopic examination was more sensitive than ZN stain.
Conclusion: One of the biggest obstacles to the correct diagnosis & efficient management of tuberculosis is the absence of rapid reporting of the bacilli in the given clinical specimen, overcome by the liquid media used in our study.

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INTRODUCTION

Care of patients with tuberculosis starts with a quality assured medical treatment. This has several facets like clinical, radiological & laboratory techniques. The laboratory techniques need to be reliable, rapid, cost effective & reproducible not only for the patients but also for the clinicians, as it will lay down the base for early initiation of suitable treatment & reduce the mortality & morbidity of the diseased person. Therefore an improved, rapid, cost effective & quality assured lab report is the need of the hour, when the world is facing the emergence of multidrug resistant tuberculosis as well as extremely drug resistant tuberculosis. (Dye *et al*.) World has 1/3rd of the population with TB. Every year there are 3 million deaths occurring worldwide, it implies that every minute a death occurs, due to this dreadful pathogen. The reemergence of this bacilli along with other illness like HIV, DIABETES makes it essential to make an early diagnosis with the clinical samples. (Anthony *et al*., 17th edition) Within the countries of the WHO, those in the east have much higher notification rate than in west (WHO 2010) Therefore TB continues to be a major public health problem & an early diagnosis is a major break through in the management & control of the disease!!

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Aim

To make an early detection & diagnosis of tuberculosis infection in clinical samples both by microscopy, culture techniques.

Objectives

1. To ensure reliable ,early detection of tuberculosis infection in clinical samples.
2. To compare two different microscopic techniques for their reliability & ease.
3. To evaluate the sensitivity & specificity of these techniques & assess the best in both methods.

4. To compare the time taken for the detection of the infection producing agent by the two culture media
5. To demonstrate the cost effectiveness of the different methods involved in the study.

To make an overall assessment of the techniques both in microscopic & cultural methods.

Inclusion criteria

Proved tuberculosis patients, patients on ATT, Fresh or newly diagnosed patients, patients who were yet to start their ATT, contacts of proved TB patients.

Exclusion criteria

Patients with other respiratory disease & infection.

MATERIALS AND METHODS

The project was submitted to the institutional ethical committee & approval was obtained – the necessary revisions were done including the title, as per the recommendations of the IEC. IEC :2014/166. After the approval, the study was started with 75 patients &25 controls. The study was done over a period of six months (May – Oct 2014). 75 Patients with the above mentioned inclusion criteria & who were willing to participate in the study after explaining the study thoroughly, were included in the study. Patients who were attending our Internal Medicine& TB CD departments were selected. Their consent was obtained & instructions for proper collection of the sputum sample were given. 25 Health care workers who were willing to participate & who were montaux negative & radiologically negative were taken as controls for this study. The sputum sample was collected in a disinfectant free, sterile container .Two samples were collected: one random & another spot .the container was duly labeled with patient name, age, sex, IP/OP no. The sputum specimens were decontaminated by trisodium phosphate & then subjected for microscopic & cultural techniques.

Microscopic method

The comparative study was done between LED microscope with AO stain & LIGHT microscope with ZN stain. The AO stain was performed as per RNTCP guidelines (Govt.of India, 2009).) & once interpreted positive, graded as per RNTCP protocol (Govt.of India, 2009). The ZN stain was done according to NCCLS protocol & examined by the light microscope, graded as per norm (Govt.of India, 2010).).

Culture method

The two culture media used for this study includes: conventional LJ MEDIA & TENDER COCONUT WATER media. The LJ media was prepared as per lab standards (Subash Chandra Parija, 2009) The tender coconut water media was prepared by filtering the tender coconut water with a gauze pad & then through whatmann filter paper -1. To the filtrate, horse serum, glycerol & pencillin was added. This was again filtered through the cellulose acetate membrane. (Vasanthakumar and Jagannath, 1998) The prepared media was ready for inoculation with the decontaminated sample. (Fig. 1) Each sample was inoculated into two media: one LJ & one tender coconut water media. They were incubated at 37 * for 6-8 weeks. The media were observed for detection of any bacterial growth on daily basis for the first week & thereafter every two days till the end of 8 weeks. The positive growth detected in the form of clumps /cords were noted & subjected for staining & confirmed. The results were tabulated & analyzed. All the culture inoculation & examination of the media for detecting the growth were done in Type II b BIOSAFETY cabinet of the MYCOBACTERIOLOGY LAB of the dept. (Fig. 2) Cost of the staining & culture methods for individual sample were also calculated & compared. Out of the 75 willing patients whose sample was subjected for staining & culture inoculation, the newly diagnosed cases were 60 in number, constituting for 80%. 10 cases were already diagnosed cases & on ATT, were attending TBCD department for further follow up, they contributed for 13%. The 5 cases constituting 6.6% were diagnosed already but yet to start on ATT. (Tab.col 1) There were 53 males & 22 females, among the willing patients, who participated in the study, giving a ratio of 2: 1. The control group comprising of HCW had 14 males & 11 females. The age group comprised mainly adults ranging from 20- 59 yrs in both sexes. The age & sex distribution is as follows: male patients:

20- 29 -4 in no-5.3%,
30-39 -11 in no- 14.6%
40-49 -15 in no -20%
50-59 -23 In no -30.6% constituting a total 53 cases.(tab.col 2)
For the female cases, it was as follows:
30-39 -7 in no -31.8%
40-49 -6 in no -27.2%
50-59 -9 in no-40.9% comprising the total of 22 patients. (tab.col 2)

The control group constituted by the HCW had the following age & sex distribution:8 males in the age group of 30-39 yrs, 6 males in the age group of 40 -49yrs making a total of 14 male volunteers. The remaining 11 controls were females with 6 ladies belonging to 30-39 yrs& 5 members with age between 40- 49 yrs.

RESULTS

Our study analysis showed that males were affected more than females, 46 males were found to be positive when compared to 27 females. The microscopic study had the following result: the conventional light microscopic examination by the Preexisting ZN stain had a positivity in 60 cases, giving 80%. While, the AO stain examined with the LED microscope proved to be fruitful in more cases -66 in no, giving 88% positivity. The culture methods were also compared: the conventional LJ MEDIA helped us to detect the mycobacterial growth on the fifth week, (Fig. 2) as many as 35 specimens had their rough, tough, buff colonies appearing after V weeks of incubation. 28 samples had their growth appearing after 6 weeks incubation, 8 specimens grew the pathogen only after 7 weeks incubation, 4 samples failed to show any growth even after 8 weeks of incubation.(tab.col 3)-94.6% In contrast to this the tender coconut water media had 56 samples becoming positive for tuberculosis colonies (Fig.3) in 10 days incubation, while 12 specimens became positive within 14 days of inoculation, while 5 specimens exhibited the growth after 21 days & 2 had contamination.(tab.col 4) -97.3%

The cost of staining the smear prepared for ZN was 20/=, whereas for AO was 30/=
Expenses incurred for culture method by LJ was found to be 100/=, for TENDER COCONUT WATER 50/=.

Table 1. Patient selection depending on Inclusion Criteria

Inclusion criteria	No. of patients
Newly diagnosed	60
Already diagnosed & on ATT	10
Diagnosed but yet to start ATT	5
Total	75

Table 2. Sex and age distribution

Age	Sex		No. of Patients
	Male	Female	
20-29	**4**	**0**	**4**
30-39	**11**	**7**	**18**
40-49	**15**	**6**	**21**
50-59	**23**	**9**	**32**
Total	**53**	**22**	**75**

Table 3. LJ media & duration of growth detection

No. of Specimens	Duration
35	>5 weeks
28	>6 weeks
8	7 weeks
4	No growth(>8weeks)

Table 4. Tender coconut water & duration of growth detection

No. of Specimens	Duration
56	10 days
28	14 days
8	21 days
2	Contaminated

Fig.1. Tender coconut water media

Fig. 2. Processing of clinical specimen in TYPE II BSC

Fig.3. LJ media with rough, tough, buff colonies

Fig.4. Tender coconut water with cord formation

DISCUSSION

Our study analysis shows that males are affected more than the females. This can be associated to the personal habits of the male like smoking, alcohol intake, which precipitates the disease more in men easily. Stress factors like peer pressure & environmental factors like occupational hazard like construction workers, also play a vital in the well being & immunity of the diseased person (Anathanarayan and Paniker's Textbook of Microbiology, 9th edition) Our college caters service to rural population, where majority of the individuals come from lower socioeconomic group, which is the most important factor for the malnourished status of the person, which in turn reduces the CMI & heralds the easy onset of Tuberculosis (Chakraborty 3rd edition). The staining technique with AO was better in comparison to the ZN stain as the former was 88% positive, while the latter was positive in 80%. The LED microscope further enhanced the study by covering larger area than in light microscope (WashingtonWinn 6th edition) 3+ samples were picked better by LED than the light microscope. Maximum of 100 fields examination was done with low/high power objectives in contrast to the 100 fields examination in oil immersion objective in Light microscope. Which was of much eye strain to the viewer. As in ZN stain there Should be as many as >10 AFB/FIELD, in LED it is > 100 AFB/FIELD to grade it as 3+. The ease of LED microscope is an added advantage in the field of lab diagnosis involving tuberculosis. This requires low power consumption, requires minimal mains & battery power. Therefore it becomes energy efficient, does not require dark room. It also gives bright & clear images. Therefore specificity is also high when compared to the light microscope. WHO recommends LED microscopes in lower income settings. (WashingtonWinn 6th edition)

This ensures the comfort & ease of the LED microscope handling

The liquid media in our study was Tender coconut water, which showed more no of isolates, 6 more than the LJ MEDIA. This proves the more sensitive nature of the liquid media used in our study. Moreover it showed the growth as early as 10th day, which confirms again the rapid nature of the culture media in contrast to the LJ MEDIA in which the growth was detected only after v weeks. When we require a rapid report it will be ideal to select a media like tender coconut water as proved in our study, which is more sensitive than the conventional LJ media. This liquid media is also cost effective than the preexisting LJ media, 50 :100/=

The control samples collected from 25 HCW were negative both by microscopy & culture methods.

Conclusion

One of the biggest obstacle to the correct diagnosis & efficient management of tuberculosis is the absence of rapid reporting of the bacilli in the given clinical specimen of the diseased patient. The current laboratory procedures that exist takes as long as 6-8 weeks to yield a specific & sensitive report. This can be overcome by the media like the one used in our study. This will ensure an early institution of therapy & improve the compliance of the patient. This will reduce the delay & prevent the development of MDR &XDR tuberculosis. As prudent medical fraternity we should remember 'Tuberculosis somewhere is tuberculosis everywhere'.

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