

A Study to Find the Utility of Modified Ziehl Neelsen Staining in the Diagnosis of Pulmonary Tuberculosis among the Pregnant Women

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Abstract

Introduction: The utility of modified Ziehl Neelsen (MZN) staining is similar to Ziehl Neelsen (ZN) staining in the diagnosis of pulmonary tuberculosis (PT). With this background, a study was conducted to find the diagnostic utility of MZN staining in the pregnant women.

Methods: It was a prospective study, conducted in the department of Microbiology, GSL Medical College. Pregnant women with diabetes mellitus (DM), symptoms of cough for > 2 weeks were considered. Non cooperative members and non DM were not considered in this research. Initially the study was explained to the study members. All the individuals were explained in local language about the importance of submission of sputum sample. Then the participants were provided with sterile, plastic containers and they were asked to collect the sample. Smear preparation, ZN and MZN were carried as per the guidelines. The data were analysed using SPSS version 21. Chi square test was used to find the statistical analysis. P <0.05 was considered to be statistically significant.

Results: Total 78 participants were included in this study, maximum in 21 – 25 years group (33.3%). Total 4 PT cases were detected and maximum in 31 – 35 (2; 50%) years group. By using ZN staining 4 (5.2%) PT cases were detected; whereas MZN also helped in the detection of these; statically there was no significant difference.

Conclusion: The diagnostic utility of MZN and ZN staining are similar. But ease in detection of acid fast bacilli is the advantage with MZN technique. Long term studies with large sample is recommended.

Keywords: Staining, Tuberculosis, Study.

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Introduction

Tuberculosis (TB) is a world pandemic caused by *Mycobacterium tuberculosis* (MTB) complex. MTB is an acid fast bacilli (AFB). Nearly 90% of TB cases occur in low and middle income countries (LMICs).

TB burden is highest in India, one-fifth of global TB burden. [1]

Sputum smear examination (SSE) is popularly used technique to demonstration

these acid fast bacilli (AFB). [2] Ease of technique, limited requirement of infrastructure are the major advantages of SSE. High TB countries such as India, SSE is a good technique for the diagnosis of TB. [3] Hence SSM is a key component of DOTS and DOTS Plus strategies for diagnosis and initiation of anti-TB treatment (ATT); in addition SSM also being used to monitor the disease progress. Hence microbiological diagnosis is the main stay for the treatment of pulmonary tuberculosis (PT). [4]

Different SSM techniques namely Ziehl Neelsen staining, modified Ziehl Neelsen (MZN) staining and so on were reported. As per Chandra TJ et al. study, the diagnostic utility of MZN staining was almost similar to that of ZN staining. [5] There is physiological diabetes in pregnant women; [6] this makes body to prone for infections. With this background, a study was conducted to find the diagnostic utility of MZN staining in the pregnant women in the diagnosis of PT.

Methods

It was a prospective study, conducted in the department of Microbiology, GSL Medical College and ACSR government Medical College. In this research sputum samples which were submitted to institutional Microscopy centre were considered. In this research, pregnant women with diabetes mellitus (DM), symptoms of cough for > 2 weeks were considered. Non cooperative members and non DM were not considered in this research.

Initially the study was explained to the study members. All the individuals were explained in local language about the importance of submission of sputum sample. Difference between sputum and saliva was shown practically. After clarifying all the doubts, the participants were explained how to produce good quality sputum sample. Simultaneously it was demonstrated practically. Then the participants were provided with sterile,

plastic containers and they were asked to collect the sample.

Collection of Sputum sample: Sputum samples were collected in an open place by coughing. Initially, patient should inhale deeply two to three times with mouth open, cough out deeply from chest, and sputum sample was spitted out directly in the sterile, new, leak-proof sample container. Then, the container was closed tightly.

Smear preparation: New unscratched slides were selected for smear preparation. Smear was prepared with sterile loop. A good smear is spread evenly, over a size of 2 × 3 cm and is neither too thick nor too thin. This was allowed to air dry for 15–30 min and fixed by passing it over a blue flame 3–4 times. [4]

ZN Staining: Smears were flooded with filtered 1% Carbol Fuchsin (CF) and heated until they were steamed and left to steam for 5 min. After rinsing the slides with a gentle stream of water, 25% sulphuric acid (H₂SO₄) was used to decolorize the smears for 2 to 4 min, and if necessary, the decolorization step was repeated for another 1–3 min. The slides were rinsed as mentioned earlier and counterstained with 0.1% methylene blue (MB) for 30 s. The slides were then washed, air dried, and examined under oil immersion. The reagents for ZN staining were prepared as per the RNTCP guidelines (1998). [4]

MZN Staining: This is very similar to that of standard ZN staining technique, except primary staining step with 1% CF was done for 15 min. [5] Smears were flooded with filtered 1% CF and heated until they were steamed and left to steam for 15 min. After rinsing the slides with a gentle stream of water, 25% H₂SO₄ was used to decolorize the smears for 2 to 4 min, and if necessary, the decolorization step was repeated for another 1–3 min. The slides were rinsed as mentioned earlier and counterstained with 0.1% MB for 30 s. The slides were then washed, air dried, and examined under oil immersion. The reagents for MZN staining

were prepared as per RNTCP (1998) guidelines.

Statistical analysis:

The data were analysed using SPSS version 21. Chi square test was used to find the statistical analysis. $P < 0.05$ was considered to be statistically significant.

Results

Total 78 participants were included in this study. Maximum number was in 21 – 25 years group (33.3%) followed by ≥ 36 years (10.3%), the mean age of the study members was 33.2 years (Table 1).

Table 1: Age wise distribution of the study participants; n (%)

Age	PT	Non PT	Total
≤ 20	0	9 (11.5)	9 (11.5)
21 – 25	0	26 (33.3)	26 (33.3)
26 – 30	1 (1.3)	18 (23)	19 (24.4)
31 – 35	2 (2.6)	14 (18)	16 (20.6)
≥ 36	1 (1.3)	7 (8.9)	8 (10.3)
Total	4 (5.2)	74 (94.8)	78 (100)

Total 4 PT cases were detected and maximum in 31 – 35 (2; 50%) years group. By using ZN staining 4 (5.2%) PT cases were detected; whereas MZN also helped in the detection of these only; statically there was no significant difference (Table 2).

Table 2: Utility of different staining techniques in the diagnosis of PT; n (%)

Technique	PT	Non PT	Total
ZN	4 (5.2)	74 (94.8)	78 (100)
MZN	4 (5.2)	74 (94.8)	78 (100)
Statistical analysis	Chi square = 0.1318; statistically not significant		

Discussion

Total 78 participants were included in this research and mean age was 33.2 years. In the current study, 33 years was the mean child bearing in this area. An Indian study by Ramadass et al. [7] reported that 29 years was the mean child bearing age for women. Ramadass et al. [7] reported study was published in 2017. In the covid era, there is lot of change in life style of human; some reports also updated regarding marital age changes. [8] This could be one of the main reasons for increase in child bearing age.

ZN staining is gold standard technique in the diagnosis of PT. it has high specificity but limited sensitivity. [4] In spite of this limitation, the WHO and RNTCP insist on smear microscopy for diagnosis and treatment of PT. [9] However, the sensitivity can be improved by providing

proper sputum sample and correct staining. [10] In this research, utmost care was taken to collect good quality sputum sample. All the participants were explained and demonstrated how to produce good quality sputum. Andrew et al. reported that more women than men produced good quality sputum (63% versus 54%). Pregnant women were the target population in this research. [11]

The second hurdle to improve the ZN sensitivity was staining technique. In this research, the staining as well as screening under microscope were carried by qualified microbiologists. Man power is the major limitation in most of the health care departments. Being a tertiary care as well as private organization, we don't face this. Moreover, in this setup we have huge number of student community to whom diagnostics is part in the curriculum.

In this research, MZN technique was compared with ZN staining. Same number of smear positive PT cases were detected, as diagnostic technique both are same and statistically there was no significant difference (Table 2). But when the smears were screened under microscope, detection of AFB was easy in the MZN stained smears. Because prolonged primary staining is the modification in MZN. This can help in screening the smears easily. But this may cause financial burden when we consider it as national programme. Hence multicentric studies with large sample size are recommended.

Conclusion

The diagnostic utility of MZN and ZN staining are similar. But ease in detection of AFB is the advantage with MZN technique. Long term studies with large sample is recommended.

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