

# Modified Ziehl Neelsen Staining in the Diagnosis of Confirmed Cases of Osteoarticular Tuberculosis

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## ABSTRACT

### BACKGROUND

Tuberculosis (TB) is a world pandemic. Osteoarticular Tuberculosis (OTB) comprises 1 - 4.3 % of all TB cases. In one of our previous reports, it was mentioned that Ziehl Neelsen (ZN) staining is a good alternative for the diagnosis of OTB. With this we studied the utility of modified ZN (MZN) staining in the diagnosis of OTB.

### METHODS

This is a cross sectional comparative study. Individuals aged > 18 years, clinically confirmed cases of OTB were included in the study. Clinical sample was collected by FNAC which was an outpatient procedure. In the microbiology laboratory, 2 smears were prepared; one was stained by ZN and other by MZN staining. Chi square test was used to find the statistical significance,  $p < 0.05$  was considered statistically significant.

### RESULTS

62 % were smear positive (SP) with ZN staining, and 67 % with MZN staining; statistically, there was no significant difference. Gender wise, with ZN staining, the smear positivity (SPT) was 36.6 %, 27 % and with MZN staining, the SPT was 39 %, 28 %, respectively for male and female; statistically there was no significant difference between the genders in both the staining techniques.

### CONCLUSIONS

MZN staining is a better technique compared to ZN staining for the diagnosis of OTB.

### KEYWORDS

Compare, Study, Staining, Tuberculosis

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**BACKGROUND**

Tuberculosis (TB) is a world pandemic, bacterial disease caused by *Mycobacterium tuberculosis* (MTB) complex (comprising MTB, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. caprae*, *M. canettii*, *M. microti*, and *M. pinnipedii*), an acid-fast bacilli.<sup>1,2,3</sup> The infection TB is one of the important causes of slow economic growth especially for high populated as well as developing countries. Loss of working days is the main cause for this financial issue due to TB.

India is the highest TB burden country in the world, the incidence is > 2 million per year. In spite of the standard diagnostic as well as treatment protocol, at free of cost, TB is spreading fast.<sup>4</sup> Infection caused by MTB complex is indistinguishable clinically, radiologically and pathologically. MTB infection to lungs i.e. pulmonary tuberculosis (PT) is very common. But MTB infection to other organs, extra PT (EPT) is also reported in the literature. Among the total TB cases, EPT accounts for 15 - 30 %.<sup>5,6</sup> Osteoarticular tuberculosis (OTB) comprises 1 - 4.3 % of all TB cases<sup>7</sup> and 10 - 15 % of all EPT cases.<sup>8</sup>

OTB most commonly occurring TB due to hematogenous seeding of the MTB.<sup>8</sup> In one of our previous reports, it was mentioned that Ziehl Neelsen (ZN) staining is a good alternative for the diagnosis of OTB.<sup>5</sup> With this we studied the utility of modified ZN (MZN)<sup>9</sup> staining in the diagnosis of OTB.

**METHODS**

This is a cross sectional comparative study conducted in the department of Microbiology, GSL Medical College, for 15 months. The individuals aged > 18 years, clinically confirmed cases of OTB were included in the study. The individuals aged < 18 years, those didn't submit the consent were excluded from the study.

From the study participants, the clinical sample was collected by FNAC which was an outpatient procedure. Under sterile precautions, the sample was collected using 20-gauge needle. For the accuracy in the diagnosis, multiple clinical specimens were collected. Immediately after collection, specimens were transported to microbiology laboratory for smear preparation, staining and for reading the stained smears. With each specimen 2 smears were prepared, one was stained by ZN staining and the other by MZN staining, respectively.

**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 X 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.<sup>10</sup>

**ZN Staining**

ZN staining was performed as per the standard guidelines.<sup>10</sup>

**MZN Staining**

This is very similar to that of standard ZN staining technique, except primary staining step with 1 % CF (Carbol Fuchsin) was done for 15 min.<sup>11</sup> 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 % H<sub>2</sub>SO<sub>4</sub> 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.

**Statistical Analysis**

Data were analysed using SPSS version 21.0. Chi square test was used to find the statistical significance, p < 0.05 was considered statistically significant.

Staining	Positive	Negative	Total
ZN	51 (62)	31 (38)	82 (100)
MZN	55 (67)	27 (33)	82 (100)
χ <sup>2</sup> Value	0.4278		
P Value	0.513561		
Analysis	Not significant		

**Table 1. Smear Results of the Study Participants n (%)**

Gender	ZN Staining		MZN Staining	
	Positive	Negative	Positive	Negative
Male	30 (36.6)	20 (24.4)	32 (39)	18 (22)
Female	22 (27)	10 (12.2)	23 (28)	9 (11)
<b>Total</b>	<b>52 (63)</b>	<b>30 (36.6)</b>	<b>55 (67)</b>	<b>27 (33)</b>
χ <sup>2</sup> Value	0.6439		0.5479	
P Value	0.422298		0.459173	
Analysis	Not significant		Not significant	

**Table 2. Gender Wise Smear Results with ZN and MZN Staining n (%)**

Age	ZN Staining		MZN Staining	
	Positive	Negative	Positive	Negative
18 - 27	3 (3.6)	4 (4.8)	4 (4.8)	3 (3.6)
28 - 37	8 (9.8)	3 (3.6)	8 (9.8)	3 (3.6)
38 - 47	12 (14.4)	6 (7.2)	13 (16)	5 (6.1)
48 - 57	16 (17)	6 (7.2)	17 (20.8)	5 (6.1)
58 - 67	6 (7.2)	8 (9.6)	7 (8.5)	7 (8.5)
> 68	6 (7.2)	4 (4.8)	6 (7.2)	4 (4.8)
<b>Total</b>	<b>51 (62)</b>	<b>31 (38)</b>	<b>55 (67)</b>	<b>27 (33)</b>
	<b>82 (100)</b>		<b>82 (100)</b>	

**Table 3: Age Wise Smear Results of the Study Participants n (%)**

**RESULTS**

During the study period, a total 82 participants who were clinically confirmed OTB was included. Among these, 62 % (51) were smear positive (SP) by ZN staining and the smear positivity (SPT) with MZN staining was 67 % (55); statistically, there was no significant difference between the staining techniques (Table 1).

Among the study participants 61 % (50) were male and 39 % (32) were female and the male female ratio was 1.56. Gender wise, with ZN staining, the SPT was 30 (36.6 %), 22 (27 %) and with MZN staining, the SPT was 32 (39 %), 23 (28 %), respectively for male and female. Statistically there was no significant difference between the genders in both the staining techniques, respectively (Table 2). Among the SP cases, the male female ratio was 1.36 and 1.4 respectively for ZN and MZN staining techniques. Age wise, with MZN staining, the SPT was 4.8 %, 9.8 %, 16 %, 20.8 %, 8.5 %, 7.2 % respectively in the age groups 18 – 27, 28 – 37, 38 – 47, 48 – 57, 58 – 67 and > 68 years. Whereas, with ZN staining, the SPT was 3.6 %, 9.8 %, 14.4 %, 17 %, 7.2 % and 7.2 %, respectively in the age groups 18 – 27, 28 – 37, 38 – 47, 48 – 57, 58 – 67 and > 68 years (Table 3).

## DISCUSSION

TB is an infection seen among the individuals with low living standards due to poverty. Being one of the high populated country's India alone accounts for 26 % of total TB cases globally<sup>12</sup> and India was declared the highest TB burden country, followed by China, Nigeria, Pakistan, Indonesia and Africa.

For the diagnosis of TB, various diagnostic methods were reported in the literature. Among these, culture on Lowenstein media is considered to be the gold standard<sup>13</sup> but requirement of prolonged time period is major limitation. Molecular techniques were also reported to be highly sensitive and specific, but here cost is the limitation hence not affordable. With these, in the current study, ZN staining was only considered for the diagnosis of OTB.<sup>14,15</sup> Several modifications of ZN were available in the literature. But in one of the study's Chandra et al. mentioned that MZN is a better diagnosis in the diagnosis of PT.<sup>9</sup> The sputum SPT was reported to be 9.43 % and 9.8 % respectively for ZN and MZN staining techniques. With these, in this study MZN staining was considered to find the SPT in the clinically diagnosed cases of OTB. In this study, the SP results were 62 % and 67 %, respectively for ZN and MZN techniques. Statistically there was no significant difference between the staining techniques (Table 1). More number (5 %; 4) of SP cases were diagnosed with MZN staining, but statistically there was no significant difference. Though statistically there was no significant difference between the ZN and MZN techniques, we should not miss even a single case. Because the patient if undiagnosed, this not only will lead to the spread of infection but also leads to financial burden, loss of working days and so on<sup>16</sup>. This ultimately will reflect on country's financial status. In the literature, the diagnostic utility of ZN was reported to be 75 %, 63 %.<sup>5, 17, 18</sup>

Gender wise, 60 % (50) were male and 39 % (32) were female participants in this study and the ratio was 1.6 (Table 2). Gender wise, with MZN staining, the SPT was 39 % (32), 28 % (23), respectively for male and female; the difference between the gender was statistically not significant (Table 2). The SPT with ZN staining was 27 %

(22), 30 (36.6 %) respectively for male and female and the difference between the gender was statistically not significant (Table 2). Among the SP cases, the male female ratio was 1.4 and 1.36, respectively for ZN and MZN techniques. As per this data, the prevalence of OTB is more among the male. Even in the literature also male dominance was reported. Sharma et al. conducted a study good quality found gender gap in the prevalence of TB with more occurrence in the rural areas compared to the urban part.<sup>19</sup> Even one of the African studies also reported higher prevalence among the male.<sup>20</sup> With these, it is clear that even OTB is common among the male. But unlike PT, gender has no influence in the diagnosis of OTB. Because in PT, the sputum submission protocol surely influences the SP results because the sample had to be produced, as well as submitted by the patient. Unlike quality of sputum, in this study, there is no influence of specimen in the diagnosis of SP OTB case because the specimen was collected by the specialist. Moreover, there was no significant financial burden with MZN technique compared to ZN technique. The minor difference in the reagents cost for MZN technique is negligible compared to the additional SP results.

Age wise, more number SP cases were diagnosed between 28 – 57 years group in both MZN and ZN techniques, which was 46.6 % and 41.3 % (Table 3). However, there was no age influence in the diagnosis of OTB using staining. Murray et al. Reported that TB is a common infection among the individuals aged 25 – 44 years<sup>21</sup>, Chinnakali et al. reported the SP TB cases were common among 20 – 54 years age<sup>22</sup>. As per these data, TB is more common among the individuals who are in active age, because this is the age for more outdoor activity for earning as well as job. So chances are more for inhalation of aerosols. Hence in this study also more cases were detected in the middle and active age group.

## CONCLUSIONS

Prevalence of OTB was more among males aged 28 – 57 years. MZN staining is a better technique compared to ZN for the diagnosis of OTB. Small sample size is the limitation of this research. Multicenter studies with a large sample size for a long duration are recommended.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

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