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Comparative Analysis of Tuberculosis Bacteria in Saliva and Sputum Using Ziehl-Neelsen, Papanicolaou and Bleach Modified Papanicolaou Induced Fluorescent Microscopy

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ABSTRACT

Background: This research contrasted the identification of Mycobacterium tuberculosis in saliva and sputum samples using Ziehl-Neelsen, Papanicolaou, and Bleach Modified Papanicolaou fluorescent microscopy.

Methodology: Sputum and saliva samples were collected in a clean sterile, leak-proof, wide-mouth containers early in the morning. Each saliva and sputum sample was further divided into 3 groups, Group A: sputum stained by Ziehl-Neelsen staining method, Group B: sputum smears stained by Papanicolaou staining method, Group C: sputum smears stained by bleach modified Papanicolaou staining method. saliva and sputum infected by Papanicolaou and observed under a Fluorescent Microscope for tubercle bacilli..

Results: The results obtained from saliva stained smears showed 63.3% of cases positive in both BMP and Pap stained saliva samples and 46.6% of cases positive in ZN stained saliva samples. Further grading of the individual staining method was carried out. Scant bacilli; 20% in Pap and 10% in BMP, 1+; 30% in Pap and 33.3% in BMP, 2+;10% in Pap and 6% in BMP and 3+-3.3% in Pap and 13% in BMP. ZN stained saliva samples scant bacilli; 56.6%, 1+; 13%, 2+; 10% and 3+; nil. Sputum samples stained by BMP, Pap, and Zn showed 100% positive results (30/30). But the grading differed among individual staining techniques.

Conclusion: In our study sputum samples showed 100% positivity in comparison to saliva samples with 63.33%. Though the results of the saliva sample were lower than that of the sputum samples, the BMP method used in our study is first of its kind to the best of our knowledge. It is a simpler, convenient, and economical method that can be used in routine diagnosis of TB.

Key words:

Mycobacterium tuberculosis, Ziehl-Neelsen, Bleach Modified Papanicolaou, Papanicolaou staining method, acid-fast bacilli. Article History:

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INTRODUCTION

Infectious diseases form a major group of ailments caused by various micro-organisms that can spread from one person to another in an environment through airborne droplets, contaminated water, food, fomites etc. The common communicable diseases found in India are Malaria, Typhoid, Jaundice, Leptospirosis, Diarrhoeal diseases, Amoebiasis, Cholera, Brucellosis, Hookworm Infection, Influenza, Filariasis, Tuberculosis (TB) etc.The infectious disease burden is high in India, because of poor sanitation, lack of access to fresh water, poor hygiene, socioeconomic status etc [1].TB has been a scourge of humanity throughout history.2&3Even after the availability of effective drugs for more than half a century, and it is still a major cause of morbidity and mortality worldwide. One-third of world's population is estimated to be infected with TB [2]. There were about 9.27 million new TB cases (including 4.1 million new smear-positive cases) and 1.3 million deaths from TB in 2008.2 There were about 11.1 million prevalent TB cases and half million MultiDrug Resistant (MDR) TB cases (resistance at least to isoniazid and Rifampcin) in the world. 95

% of TB cases and 98% of TB deaths occur in developing and under developed countries.^{2,3} It is leading cause of death affecting the productive segment of population [3]. It is the sixth most common cause of mortality (2.5%) next to HIV infection and accounts for 26% of preventable deaths in the world [1,3,4,5]. TB is a disease caused by a bacterial agent: Mycobacterium tuberculosis (MTB). It is an infectious disease that is transmitted from person to person through the air via aerosol when a patient expels bacteria by coughing, sneezing, spitting etc. The spread of the organismis affected by multiple factors including duration of exposure, infectiousness of patient, host factors and environmental conditions [1]. Only those with active form of pulmonary TB are infectious, with the translation of latent to active form depending primarily on the host's immunity. Although the asymptomatic latent form of TB is non-infectious, once progressed to an active form, it is said to kill more than 50% of patients if left untreated [5,7]. Tuberculosis is a global health concern with approximately 9 million people developing an active, contagious form every year [8]. About 40% of the Indian population is infected with TB bacteria that chiefly affect lungs other organs like lymph

nodes, bones, central nervous system are also affected [2]. The infectious dose is approximately 1-200 bacilli, but each aerosol droplet can contain 1-400 bacilli [2]. Upon inhalation, the bacilli lodge into the alveoli where it multiply and form a primary lesion [6]. Under normal condition, in most of the cases, the immune system either clears the bacilli or arrests the growth of the bacilli within the primary lesion in which case the host is said to harbor latent TB infection (LTBI) [2]. However, in 5 - 10% of the cases, the bacilli overwhelm the immune system resulting in a primary TB within a few months to years. In the rest, post-primary TB occurs when re-infection occurs or the LTBI is reactivated. The lifetime risk of developing active TB is 5 - 10 %. It could be higher because of the underlying conditions HIV infection, diabetes and other medical conditions that suppress immunity [2,3]. Tuberculosis bacteria travels to the lungs and harbors in the aveoli, which activate body's defense mechanism. The fore most signs and symptoms of tuberculosis include sputum, haemoptysis, breathlessness, weight loss, anorexia, fever, malaise, wasting, and terminal cachexia [1,3,5,6]. The major strategy for case finding has been passive, whereby people with cough and other related symptoms report to health facility for further investigation. TB diagnostic tests are available although no single diagnostic test for TB exists that can be performed rapidly, simply, inexpensively, and accurately as a stand-alonetest. Thus, the diagnosis of active TB is a clinical exercise; and sputum microscopy remains the mainstay of diagnosis because of its availability, operational feasibility and ability to identify the highly infectious forms of TB [2-7]. The significance of TB diagnosis is high if and only if it is complemented by prompt treatment. If not treated in the earliest five years, 50% of PTB cases die, 25% self cure and 25% remain sick and infectious [3]. Untreated smear-positive PTB patient can infect 10 - 15 people per year on an average [15-17]. Thus, treatment of TB is not only a matter of treating the individual patient, but also is an important public health intervention. Treatment is the centerpiece of TB control and can reduce the risk of infection if implemented with improving adequate coverage and acceptable quality. 16,17 So far, Directly Observed Treatment, Short Course (DOTS) remains a cost-effective intervention to control TB [1]. Diagnosis of TB among suspects is conventionally based on sputum smear microscopy, culture, followed by chest X-ray for confirmation [3,5,7]. Early diagnosis and treatment of Tuberculosis is crucial for reduction of infection rate and improving outcome of the treatment. Despite efforts made for early detection of TB still there is a significant delay in healthcare seeking among TB patients [2]. There are four types of delay in tuberculosis management: (a) Patient delay (b) Health care, (c) Diagnostic delay and (d) Treatment delay[2]. The diagnosis of TB rests on the identification of *Mycobacterium* in samples taken from patients. These methods can be broadly categorized under three headings: culture, microscopy and molecular methods [1,3,5,7]. Culture is the gold standard for identification of tubercle bacilli. Various culture methods are available today which includes solid media such as Lowenstein-Jensen (LJ) medium to liquidbased techniques and automated radiometric and nonradiometric systems [1-5]. However the process of microbial culture is laborious, very slow and takes three to approximately 14 days for results [1-7]. Microscopic examination is an essential element of the bacteriological diagnosis of tuberculosis. The specimens used are usually sputum samples, bronchial brushing, lavage or aspiration

preparations [6-17. This method is simple, cost-effective and provides rapid results but suffers from lack of sensitivity in case of poor quality sputum which is common in case of extra pulmonary tuberculosis, TB in children and HIV-co infected patients. Saliva an alternative diagnostic sample is easy to obtain and process, with an extra advantage of being used in case of patients with non productive [5-7]. Ziehl-Neelsen (ZN) is the most extensively used procedure for the demonstration of Mycobacterium tuberculosis in smears as it is believed to be simple, cost effective, quick and effective method for diagnosis. The major disadvantage is lowered sensitivity and the smear remains infectious while being handled in laboratory. Various attempts have been done to increase the sensitivity with use of Fluorescent microscope.8-17 The flurochrome staining is regarded as a sensitive and reliable method for detection of tubercle bacilli. Most commonly Auramine-Rhodamine [A-R], is used in patients with low density of bacilli that are likely to be missed on ZN stained smears. There is a strong binding of phenol auramine with mycolic acid of tubercle bacilli which when observed under fluorescent microscope glow and help in identification under high power (40x). false positive results may also occur due to staining of inanimate objects, the sample remains infectious while being handled in laboratory and also the cost of the stains hinders its use in routine practice [24-42]. Papanicolaou (Pap) stain is widely used in routine cytological evaluation of samples. Its value in fluorescent microscopy for diagnosis of tuberculosis is well established. In Papanicolaou stained smears under fluorescent microscope tubercle bacilli is readily identifiable. The staining technique can be used routinely as it is simple, sensitive, safe and easy. However the disadvantage is that debris and overcrowding of cells in the smears interfere during diagnosis, also the sputum or saliva sample remain infectious while being handled in laboratory [24-27]. Liquefaction of sputum or saliva by sodium hypochlorite and concentration of bacilli through centrifugation will significantly increase the sensitivity of bacilli, treating the sample with 2 to 5% of sodium hypochlorite clears debris and other cells, and helps in identification of the bacilli even in the very low load as, NaOCl causes changes in the surface properties of acid fast bacilli (i.e., charge and hydrophobicity) and/or denaturation of the specimen leading to flocculation and subsequent increased sedimentation of the acid fast bacilli.18 Furthermore, the increased smear positivity by bleach method is attributable to the higher density of bacilli per microscopic field thus it reduces the time required to detect acid fast bacilli [12-37]. Use of this method would definitely lower the risk of laboratory infection as sodium hypochlorite kills the Mycobacterium [16-21]. There is a wide gap between the technologies being developed and their use in routine practices. The transition from development to widespread application depends on social, scientific, political and financial factors. Based on these concerns the aim of this work was development of safer, accurate, quick and affordable diagnostic staining for TB that will yield results at a faster pace.

MATERIALS AND METHODS

The present study was conducted in the Department of Oral Pathology and Microbiology, Farooqia Dental College and Hospital, Mysore. Sputum and Saliva samples of confirmed Pulmonary Tuberculosis cases were collected from TB Sanatorium Mysore. Sample sputum -30, saliva -30. Sputum and saliva samples were collected in a clean sterile, leak proof, wide mouth containers early in the morning. Each saliva and

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sputum sample were further divided into 3 groups, Group A: sputum stained by Ziehl-Neelsen staining method, Group B:sputum smears stained by Papanicolaou staining method, Group C:sputum smears stained by bleach modified Papanicolaou staining method. saliva and sputum infected by Papanicolaou and observed under a Fluorescent Microscope for tubercle bacilli. Slides stained by ZN, AFB were demonstrated by oil immersion lens using 100X magnification, bacilli appeared as red rods against blue background under light microscope, Slides stained by Pap, AFB were demonstrated using 40x magnification, bacilli appeared fluorescent yellow against dark background under fluorescent microscope, Smears of saliva and sputum samples treated with bleach were stained by Pap, AFB were demonstrated using 40x magnification; bacilli appeared as fluorescent yellow rods against green background under fluorescent microscope.

STATISTICAL ANALYSIS

Data analysis was performed using the statistical analyzed using SPSS software and descriptive statistics like mean and standard deviation were calculated. Cramer's V test was done to find out association between the groups used as inferential statistics. P < 0.005 is considered statistically significant.

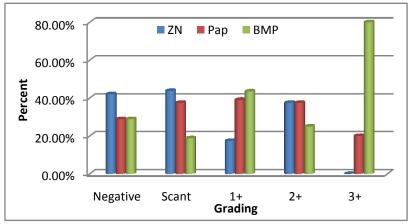
RESULTS

The results obtained from saliva stained smears showed 63.3% of cases positive in both BMP and Pap stained saliva samples and 46.6% of cases positive in ZN stained saliva samples. Further grading of individual staining method was carried out. Scant bacilli; 20% in Pap and 10% in BMP, 1+; 30% in Pap and 33.3% in BMP, 2+;10% in Pap and 6% in BMP and 3+-3.3% in Pap and 13% in BMP. ZN stained saliva samples scant bacilli; 56.6%, 1+; 13%, 2+; 10% and 3+; nil. Sputum samples stained by BMP, Pap and Zn showed 100% positive results (30/30). But the grading differed among individual staining techniques. Scant; 26.6% in Zn, 10% in Pap, and 3.3% in BMP. 1+; 30% in ZN, 10% in Pap and 16.6% in BMP. 2+; 30% in ZN, 40% in Pap and 33.3% in BMP. 3+; 13.3% in ZN, 40% in Pap and 46.6% in BMP. BMP stained sputum and saliva samples showed maximum bacilli per field in comparison Pap and ZN.

Table 01: Comparison of Smear results obtained using ZN, Pap and BMP staining techniques in saliva samples

Zn, Pap and BMP staining technique in saliva samples					
	RADING		GROUPS		Total
G	KADING	ZN	Pap	ВМР	Total
****	Frequency	16	11	11	38
-ve	%	42.1%	28.9%	28.9%	100.0%
Coant	Frequency	7	6	3	16
Scant	%	43.8%	37.5%	18.8%	100.0%
1+	Frequency	4	9	10	23
1+	%	17.4%	39.1%	43.5%	100.0%
2+	Frequency	3	3	2	8
Δ+	%	37.5%	37.5%	25.0%	100.0%
2.	Frequency	0	1	4	5
3+	%	0.0%	20.0%	80.0%	100.0%
Total	Frequency	30	30	30	90
Total	%	33.3%	33.3%	33.3%	100.0%

		Value	Approx. Sig.
Nominal by Nominal	Cramer's V	.248	.197



Graph 01: Grading of ZN, Pap and BMP stained saliva smears in percent.

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Among the 3 stains maximum of 80%, 3+ grading was seen in BMP followed by Pap; 20% and ZN; 0% stained saliva smears.

Maximum of 37.5%, 2+ grading was seen in ZN and Pap respectively followed by BMP; 25% stained saliva smears.

Maximum of 43.5%, 1+ grading was seen in BMP followed by Pap; 39.1% and ZN; 17.4% stained saliva smears.

Maximum of 43.8% scant grading was seen in ZN followed by Pap; 37.5% and BMP; 18.8% stained saliva smears.

Maximum of 42.5% negative grading was seen in ZN followed by Pap and BMP 28.9% each, stained saliva smears.

or 12.0 % negative grading was seen in 2.0 femous day 1 ap and 2.00 % each, stained survive sinears.

Table 02: Comparison of Smear results obtained using ZN and Pap staining techniques in saliva smears

ZN VERSUS PAP STAINING - SALIVA				
CD	GRADING		OUP	TOTAL
G.			Pap	TOTAL
***	Frequency	16	11	27
-ve	%	59.3%	40.7%	100.0%
Scant	Frequency	7	6	13
	%	53.8%	46.2%	100.0%
1	Frequency	4	9	13
1+	%	30.8%	69.2%	100.0%
2+	Frequency	3	3	6
2+	%	50.0%	50.0%	100.0%
2.	Frequency	0	1	1
3+	%	0.0%	100.0%	100.0%
Total	Frequency	30	30	60
	%	50.0%	50.0%	100.0%

	Value	Approx. Sig.	
Nominal by Nominal	Cramer's V	.256	.416

Table 02: Comparison of smear results obtained using ZN and Pap staining techniques in saliva smears with P value of 0.416.

Table 03: Comparison of Smear results obtained using ZN and BMP staining techniques in saliva

	ZN VI	ERSUS BMP STAINING - S	SALIVA	
GRADING		GRO	OUP	TOTAL
ur	ADING	ZN BMP		TOTAL
***	Frequency	16	11	27
-ve	%	59.3%	40.7%	100.0%
Scant	Frequency	7	3	10
Scalit	%	70.0%	30.0%	100.0%
1	Frequency	4	10	14
1+	%	28.6%	71.4%	100.0%
2+	Frequency	3	2	5
Δ+	%	60.0%	40.0%	100.0%
2.	Frequency	0	4	4
3+	%	0.0%	100.0%	100.0%
Total	Frequency	30	30	60
	%	50.0%	50.0%	100.0%

		Value	Approx. Sig.
Nominal by Nominal	Cramer's V	.394	.054

Table 03: Comparison of smear results obtained using ZN and BMP staining techniques in saliva smears with a P value of 0.054.

Table 04: Comparison of Smear results obtained using Pap and BMP staining techniques in saliva

Pap versus BMP staining – saliva				
C 1!		Group		Total
u	rading	Pap	BMP	Total
****	Frequency	11	11	22
-ve	%	50.0%	50.0%	100.0%
Scant	Frequency	6	3	9
	%	66.7%	33.3%	100.0%
1+	Frequency	9	10	19
	%	47.4%	52.6%	100.0%

2+	Frequency	3	2	5
Δ+	%	60.0%	40.0%	100.0%
2⊥	Frequency	1	4	5
3+	%	20.0%	80.0%	100.0%
Total	Frequency	30	30	60
	%	50.0%	50.0%	100.0%

		Value	Approx. Sig.
Nominal by Nominal	Cramer's V	.226	.549

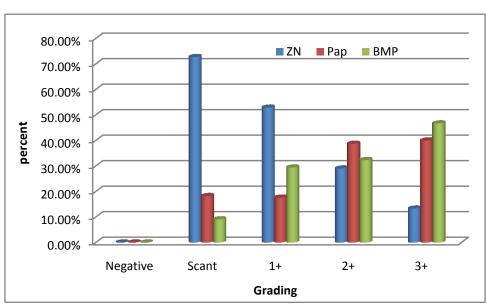
Table 04: Comparison of smear results obtained using Pap and BMP staining techniques in saliva with a P value of 0.054.

Table 05: Comparison of smear results obtained using Zn, Pap and BMP staining techniques in sputum smears

	ZN, PAP AND BMP S	STAINING TECHN	IQUE IN SPUTUM S	MEARS	
CDADING		GROUPS			тоты
Gr	RADING	ZN	PAP	BMP	TOTAL
Cant	Frequency	8	3	1	12
Scant	%	72.7%	18.2%	9.1%	100.0%
1.	Frequency	9	3	5	17
1+	%	52.9%	17.6%	29.4%	100.0%
2+	Frequency	9	12	10	31
2+	%	29.0%	38.7%	32.3%	100.0%
3+	Frequency	4	12	14	30
3+	%	13.3%	40.0%	46.7%	100.0%
Total	Frequency	30	30	30	90
	%	33.3%	33.3%	33.3%	100.0%

		Value	Approx. Sig.
Nominal by Nominal	Cramer's V	.326	.014

Table 05: Comparison of smear results obtained using Zn, Pap and BMP staining techniques in sputum smears with a P value of 0.014.



Graph 02: Grading of ZN, Pap and BMP stained sputum smears in percent.

Among the 3 stains maximum of 46.7%, 3+ grading was seen in BMP followed by Pap; 40% and ZN; 13.3% stained sputum smears. Maximum of 38.7%, 2+ grading was seen in Pap followed by BMP; 32.3% and ZN; 29% stained sputum smears. Maximum of 52.9%, 1+ grading was seen in ZN followed by Pap; 17.6%% and ZN; 29.4% stained sputum smears. Maximum of 72.7% scant grading was seen in ZN followed by Pap; 18.2% and BMP; 9% stained sputum smears. 0% negative grading was seen in ZN, Pap and BMP stained sputum smears.

Table 06: Comparison of smear results obtained using ZN and Pap staining techniques in sputum

ZN VERSUS PAP STAINING - SPUTUM				
CDADING		GR	OUP	TOTAL
GK.	GRADING		ZN PAP	
Scant	Frequency	8	3	11
Scalit	%	80.0%	20.0%	100.0%
1+	Frequency	9	3	12
1+	%	75.0%	25.0%	100.0%
2+	Frequency	9	12	21
2+	%	42.9%	57.1%	100.0%
3+	Frequency	4	12	16
3+	%	25.0%	75.0%	100.0%
Total	Frequency	30	30	60
I Otal	%	50.0%	50.0%	100.0%

Symmetric Measures			
		Value	Approx. Sig.
Nominal by Nominal	Cramer's V	.448	.017

Table 06: Comparison of smear results obtained using ZN and Pap staining techniques in sputum with a P value of 0.017.

Table: 07 Comparison of smear results obtained using ZN and BMP staining techniques in sputum

	ZN VEF	RSUS BMP STAINING - S	PUTUM	
CD	GRADING		GROUPS	
GRADING		ZN	ВМР	TOTAL
Scant	Frequency	8	1	9
	%	88.9%	11.1%	100.0%
1+	Frequency	9	5	14
	%	64.3%	35.7%	100.0%
2+	Frequency	9	10	19
	%	47.4%	52.6%	100.0%
3+	Frequency	4	14	18
	%	22.2%	77.8%	100.0%
Total	Frequency	30	30	60
	%	100.0%	100.0%	100.0%

		Value	Approx. Sig.
Nominal by Nominal	Cramer's V	.451	.007

Table 07: Comparison of smear results obtained using ZN and BMP staining techniques in sputum with a P value of 0.007.

Table 08: Comparison of smear results obtained using Pap and BMP staining techniques in sputum

PAP VERSUS BMP STAINING - SPUTUM					
CD	ADING	GROUP		TOTAL	
GK	ADING	PAP	ВМР	TOTAL	
Scant	Frequency	3	1	4	
Scant	%	66.7%	33.3%	100.0%	
1+	Frequency	3	5	8	
1+	%	37.5%	62.5%	100.0%	
2+	Frequency	12	10	22	
Δ+	%	54.5%	45.5%	100.0%	
3+	Frequency	12	14	26	
	%	46.2%	53.8%	100.0%	
Total	Frequency	30	30	60	
iotai	%	50.0%	50.0%	100.0%	

		Value	Approx. Sig.
Nominal by Nominal	Cramer's V	.190	.705

Table 08: Comparison of smear results obtained using Pap and BMP staining techniques in sputum with a P value of 0.705

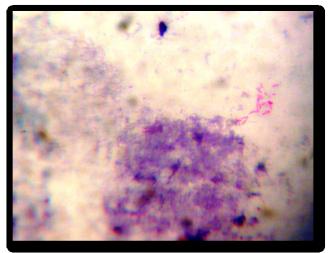


Figure 04: AFB seen as bright red rods against blue background, occurring in small groups, (100x) under light microscope in ZN stained saliva smears



Figure 05: AFB seen as bright red rods against blue background, occurring singly, in pairs and as small groups, (100x) under light microscope in ZN stained sputum smears.

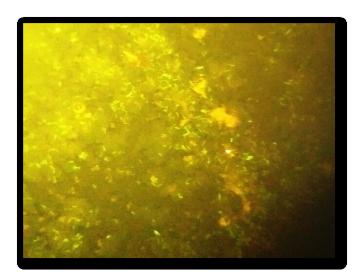


Figure 06: Fluorescent bacilli appearing as slender yellow green straight or curved rods of relatively uniform length against dark background (40x) stained by Pap under fluorescent microscope in saliva sample.

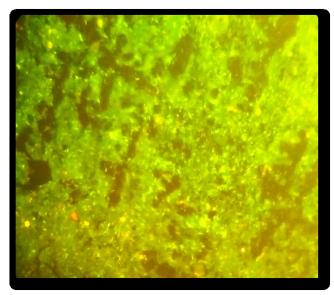


Figure 07: Numerous fluorescent bacilli appearing as slender yellow green straight or curved rods of relatively uniform length against dark background, clumping and crowding of epithelial and mucous cells masking the fluorescence of AFB (40x) stained by Pap under fluorescent microscope in sputum sample.

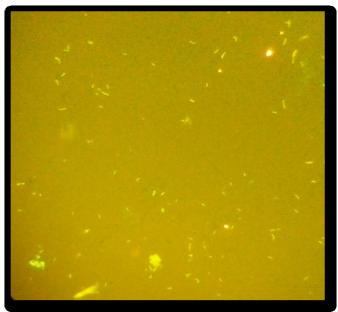


Figure 08: Fluorescent bacilli appearing as slender yellow green straight or curved rods of relatively uniform length against clear background (40x) stained by BMP under fluorescent microscope in saliva sample.

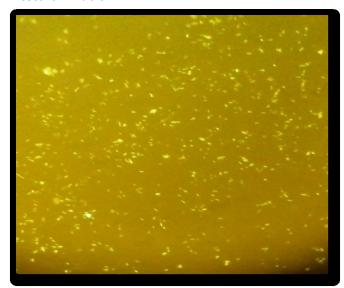


Figure 09: Numerous fluorescent bacilli appearing as slender yellow green straight or curved rods of relatively uniform length against clear background (40x) stained by BMP under fluorescent microscope in sputum sample.

DISCUSSION

Tuberculosis is an infectious granulomatous lesion caused by Mycobacterium tuberculosis. It is highly aerobic and requires high levels of oxygen to survive. M. Tuberculosis is primarily a pathogen of respiratory system which most commonly infects the lungs. the cells impervious to commonly used staining methods in identification of bacteria including Gram stain. Hence conventional ZN stain or acid-fast stain is routinely used. According to the WHO guidelines for TB control, The conventional ZN staining is simple, rapid and economical diagnostic method. But this method requires high amount of organisms in the specimen. Hence the sensitivity of this method ranges from 20% to 43%. A study by Holani et al [24]. have successfully demonstrated the presence of tubercle bacilli in saliva using flurochrome staining. Saliva samples when aided with flurochrome has overcome the difficulties encountered with sputum smear microscopy like lack of adequate sputum in patient with non-productive cough, cumbersome process involved in collection and processing of the sputum. Further, its direct relevance to dentistry prompted its use in the present study. Our study was conceptualized in order to overcome these drawbacks of conventional ZN sputum smear microscopy. In the present study sputum and saliva samples were obtained from clinically suspected cases of pulmonary tuberculosis and were stained using ZN, Pap and BMP techniques. Both saliva and sputum stained smears were then scanned as per grid pattern proposed by National TB Institute [17,24]. ZN stained slides were observed under light microscope and Pap and BMP stained slides were observed under fluorescent microscope respectively. In our study saliva stained smears showed 63.3% of positive cases in both BMP and Pap stained samples under fluorescent microscope and 46.6% of cases were positive in ZN stained saliva samples when observed under light microscope. The above saliva stained smears were further graded individually. Our results showed: Scant bacilli; 20% in Pap and 10% in BMP, 1+; 30% in Pap and 33.3% in BMP, 2+;10% in Pap and 6% in BMP and 3+;3.3% in Pap and 13% in BMP. ZN stained saliva samples showed scant bacilli in 56.6%, 1+ in 13%, 2+ in 10% and 3+

was nil. Among the three staining techniques used in the detection of *M. tuberculosis* in saliva samples, BMP stained slides under fluorescent microscopy identified maximum number of bacilli per field. When ZN and Pap stained saliva samples were compared, ZN stained samples showed 46.6% positive cases and Pap showed 63.3% positive cases. In our study using sputum samples, maximum grading of 3+ was observed in BMP (46.7%) followed by Pap (40%) and minimum in ZN (13.3%). In our study, the results of saliva and sputum samples were also compared. 100% positivity was observed in ZN, Pap and BMP stained sputum samples and 63.33% positive cases were identified in saliva samples. Studies by Lunawth P et al.²⁴ and Honali et al [23]. showed 100 % positivity in sputum samples stained by ZN and flurochrome which were in concordance with our sputum results. BMP method used in our study is first of its kind. It is a simpler, convenient and economical method that can be used in routine diagnosis of TB. Additional analyses with a larger sample size are needed to enhance the accuracy and use of saliva tests.

CONCLUSION

The aim of the present study was to identify *M.Tuberculosis* in the sputum and saliva smears using Pap and BMP and compare its efficacy over routinely used ZN staining technique. our study showed that BMP is safe, easy to perform, faster results and cost effective which can be employed in routine practice. Samples when stained by BMP showed maximum number of bacilli per field in comparison to ZN and Pap. The use of fluorescent microscope over light microscopy has shown superior results. Dentists and their assistants are at higher risk of acquiring Tuberculosis, due to frequent exposure to infectious droplets from patients while performing various dental procedures. Specific grading system for Pap and BMP induced fluorescence is required in order to maximize utility of this technique.

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FINANCIAL DISCLOSURE

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FUNDING

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CONFLICT OF INTEREST

The authors have indicated they have no potential conflicts of interest to disclose.

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