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Microscopic Quality Evaluation of Sputum Specimens Submitted for Diagnosis of Tuberculosis at the TB Clinic, University of Uyo Teaching Hospital, Akwa Ibom State, Nigeria.

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Abstract

The quality of sputum specimen may be determined using Gram stain and this predicts likely pathogens. Determining the quality of the specimen is based on the numbers of polymorphonuclear leucocytes and squamous epithelial cells (SECs) present. The Sputum specimens were first processed using standard Ziehl-Neelsen (ZN) staining for AFB, after which the samples were Gram-stained and examined microscopically under low power (10X); the average number of squamous epithelial cells and inflammatory cells per low-power field (LPF) was estimated from ten fields. Out of 130 sputum samples processed, Females accounted for the highest number of AFB positive smear results 10(52.6), 13(61.9) respectively, while males accounted for the lowest AFB negative smear results 9(47.4), 8(38.1) respectively in each of the samples. In sample 1, the age range 24-39 had the highest 28(25.2) and 6(31.6) both in negative and positive smear results respectively. In Sample 2 and 3, the age range 40-49 had the highest 7(33.3) positive smear results. A total of 91(70.0%) sputum samples were below the standard criteria, by Gram stain, showing <10 pus cells and >25 epithelial cells, while 39(30.0%) showed the standard criteria with <25 epithelial cells and >10 pus cells from Sample 1, Sample 2 and Sample 3. (Table 1) Early morning specimens were seen to have 100% AFB positive detection rate throughout Table 1 and 2. This may be due in part to the accumulation of sputum in the lungs overnight, resulting in a concentration of bacilli in the early morning samples.

Keywords: Acid-Fast Bacilli (AFB), squamous epithelial cells (SECs), Pus cells.

Introduction

Sputum specimens are among the most frequently rejected by microbiology laboratories because of a failure to meet minimum standards set by quality control (Bartlett, R. C., 1974). Some authorities even suggest that little is to be gained by processing sputum specimens (Kumar V et al, 2007). Others comment on the unreliability of attempting to diagnose pneumococcal pneumonia from gram-stained smears or cultures (Hahn, H. H., and H. N. Beatty, 1970).

According to the pulmonary tuberculosis (TB) guidelines, patients require instructions regarding the proper method of sputum collection. Patients need to be informed that a desired sputum specimen consists of material brought up from the lungs after a productive cough and not nasopharyngeal discharge or saliva (Yeon Joo Lee *et al*., 2015)

To ensure reliable, high quality laboratory services, quality assurance of sputum microscopy is essential. The purpose of a quality assurance system is the improvement of efficiency and reliability of smear microscopy services. It must be stressed here, that quality assurance has nothing to do with the diagnosis

or the clinical management of the individual TB patient. The DOTS strategy also recommends that good quality sputum smear microscopy should be an integral part of any TB control programme as it is the primary diagnostic tool for such programmes, as well as being relatively less expensive and technologically simple (RNTCP, 1998).

Several microscopic sputum quality assessment criteria have been developed since the 1970s, aimed primarily at assessing sputum quality (Mishal S. K *et al*., 2009). As squamous epithelial cells (SECs) are only found in the upper respiratory tract, the amount of SECs in a specimen is an indicator of oropharyngeal contamination (Wong et al. 1982), and can be used to indicate whether the specimen originates from deep within the lungs.

Those with large numbers of squamous epithelial cells are presumed to contain a large volume of oropharyngeal flora that would obscure or misrepresent microbiologic findings associated with lower respiratory tract infections. The further quality of sputa is evaluated according to the number of inflammatory cells, especially polymorphonuclear leukocytes (PMNs). The presence of these inflammatory cells (suggesting acute inflammation) is considered to be associated with "better" specimens (Q-probes, 1991).

Attempts have been made to eliminate contamination by upper respiratory flora by using methods which include transtracheal (TT) aspiration, tracheal puncture, bronchoscopy, needle aspiration of the lung, and lung biopsy (Murray, P. R., and J. A. Washington H, 1975). However, these may be impractical for routine practice and may be accompanied by considerable risk, including death (Schillaci, R. F., and V. E. Iacovoni, 1976).

Recent literature described methods for categorizing sputa after examination of a stained smear. Murray and Washington (1975) screened sputa according to the numbers of squamous epithelial cells (SEC) observed at a magnification of x100 and found that the mean number of bacterial species isolated was greater than four in specimens which contained more than 10 SEC per field. In another method the sputum is given a rating number in accordance with the quantitative presence of polymorphs, mucus, and SEC (Bartlett, R. C., 1974).

The best sputum samples contain very little saliva, as this contaminates the sample with oral bacteria. Then for squamous epithelial cell, more than 25 of SEC at low enlargement indicates saliva contamination (Nur Shahida B, N., 2012)

Our investigations were done as same described by R S Martin (1978) which covered a period of 6 months. Initially, the screening method applied was that described by Murray and Washington. This was superseded by the differential method described here in which both polymorphonuclear cells (PNC) and SEC were used.

Materials and Methods

Patients presented at the TB clinic of University of Uyo teaching hospital with respiratory symptoms of more than 3 weeks' duration, were screened for TB and The sample collection was done under NTP conditions, the IUATLD recommends collecting three sputum samples for TB suspects "on the SPOT – early MORNING – on the SPOT", preferably within two days. These samples were examined by smear microscopy at the Mycobacterium laboratory of University of Uyo teaching hospital.

AFB (Acid Fast Bacilli test) staining/ smear Microscopy

The Sputum specimens were first processed using standard Ziehl-Neelsen (ZN) staining and sputum smear microscopy techniques to determine their smear status. Acid Fast Bacilli were observed under microscope following the ZeihlNeelsen staining technique. Uniform and consistent smear were prepared with loop taking purulent portion of sputum sample and heat fixed. Staining was performed as per standard protocol of Ziehl-Neelsen staining method (Tortora G J, 2007). The results were noted down as per observation under microscope.

A smear of each specimen was Gram-stained and examined microscopically under low power (10X); the average number of squamous epithelial cells and inflammatory cells per low-power field (LPF) was estimated from ten fields.

Results

Of 130 sputum samples processed in microbiology lab of University of Uyo teaching hospital during the period of 6months, 73(56.2) sputum samples were submitted by females while 57(43.8) were submitted by males. Females accounted for the highest number of AFB positive smear results 10(52.6), 13(61.9)

respectively, while males accounted for the lowest AFB negative smear results 9(47.4), 8(38.1) respectively in each of the samples. Sample 1 had the highest 111 negative AFB smear results but the lowest 19 positive AFB smear results. Sample 2 and 3 had the lowest 109 each, AFB negative smear results but highest 21 each, AFB positive smear results. In sample 1, the age range 24-39 had the highest 28(25.2) and 6(31.6) both in negative and positive smear results respectively. In Sample 2 and 3, the age range 40-49 had the highest 7(33.3) positive smear results. A total of 91(70.0%) sputum samples were below the standard criteria, by Gram stain, showing <10 pus cells and >25 epithelial cells, while 39(30.0%) showed the standard criteria with <25 epithelial cells and >10 pus cells from Sample 1, Sample 2 and Sample 3. (Table 1)

Table 2 shows the total number of Sample 1,2 and 3 positive smears observed according to the recommended grading (1+,2+,3+) from the criteria for acceptance.

Table 1: Characteristics of sputum specimens results among TB suspects

Characteristics	AFB Results						Total Enrolled n(%)
	Sample 1 (Spot)		Sample 2 (Early Morning)		Sample 3 (Spot)		
	Neg n(%)	Pos n(%)	Neg n(%)	Pos n(%)	Neg n(%)	Pos n(%)	
Total	111	19	109	21	109	21	130
Sex							
Male	48(43.2)	9(47.4)	49(45.0)	8(38.1)	49(45.0)	8(38.1)	57(43.8)
Female	63(56.8)	10(52.6)	60(55.0)	13(61.9)	60(55.0)	13(61.9)	73(56.2)
Age Ranges							
2-14	10(9.0)	1(5.3)	10(9.2)	1(4.8)	10(9.2)	1(4.8)	11(8.5)
15-19	16(14.4)	1(5.3)	16(14.7)	1(4.8)	16(14.7)	1(4.8)	17(13.1)
20-24	18(16.2)	2(10.5)	17(15.6)	3(14.3)	17(15.6)	3(14.3)	20(15.4)
25-39	28(25.2)	6(31.6)	28(25.7)	6(28.6)	28(25.7)	6(28.6)	34(26.2)
40-49	23(20.7)	6(31.6)	22(20.2)	7(33.3)	22(20.2)	7(33.3)	29(22.3)
50+	16(14.4)	3(15.8)	16(14.7)	3(14.3)	16(14.7)	3(14.3)	19(14.6)
Criteria for acceptable specimen							
(WBC<10/LPF, EPC≥25/LPF)	90(81.1)	1(5.3)	91(83.5)	0(0.0)	90(82.6)	1(4.8)	91(70.0)
(WBC>10/LPF, EPC<25/LPF)	21(18.9)	18(94.7)	18(16.5)	21(100)	19(17.4)	20(95.2)	39(30.0)

Table 2: AFB positive grading from spots and early morning sputum samples by Murray & Bartlett criteria

Criteria for acceptable specimen	Results	Sample 1 (Spot) n=19(%)	Sample 2 (Early Morning) n=21(%)	Sample 1 (Spot) n=21(%)
(WBC<10/LPF, EPC≥25/LPF)	1+	1(5.3)	0	1(4.8)
	2+	0	0	0
	3+	0	0	0
(WBC>10/LPF, EPC<25/LPF)	1+	5(26.3)	6(28.6)	6(28.6)
	2+	7(36.8)	8(38.1)	7(33.3)
	3+	6(31.6)	7(33.3)	7(33.3)

Discussion

Gram stains on sputum specimens may be used for determining the quality of the specimen and for predicting likely pathogens. Determining the quality of the specimen is based on the numbers of polymorphonuclear leucocytes and squamous epithelial cells (SECs) present.

From the standard criteria for good sputum specimen quality (WBC>10/LPF, EPC<25/LPF), It was seen that Sample 1, 18(94.7), Sample 2, 21(100) and Sample 3, 20(95.2) had the highest AFB positive smear results as against the wrong sputum quality (WBC<10/LPF, EPC≥25/LPF) which had one or no AFB positive smear result in sample 1, 2 and 3 (Table 1). A study by Beena P J (2009) agrees with these findings which out of 2069 expectorated sputum samples analyzed, 1440 (69.5%) sputum samples were seen to be below the standard criteria, by Gram stain, showing more epithelial cells and scanty pus cells while only 629 (30.5%) sputum samples were of good quality with the standard criteria of <10 epithelial cells and >25 pus cells.

Early morning specimens were seen to have 100% AFB positive detection rate throughout Table 1 and 2. This may be due in part to the accumulation of sputum in the lungs overnight, resulting in a concentration of bacilli in the early morning samples.

The quality of sputum specimen collection needs to be improved by many institutions. Many laboratories could also do more to deal with the diagnostic limitations of this type of specimen, and decrease the potential of misinterpretation of results. For example, according to the recommended grading guidelines, from the criteria (WBC<10/LPF, EPC≥25/LPF) it was seen that at 1+ samples 1 and 2 which were the spots had 1(5.3) AFB positive result but sample 2 which is the early morning sample had 0(0.0) AFB positive result. In contrast, this might be due to the fact that patients may be more active during the day and may shed bacilli intermittently, thus reduce the yield of bacilli in spot sputum samples.

In (WBC>10/LPF, EPC<25/LPF) with regards to the recommended TB grading guideline, the results were seen to be showing differences in the result grading. In sample 2, 1+,2+,3+ results changed from what was obtained in sample 1, from 5(26.3) to 6(28.6), 7(36.8) to 8(38.1), 6(31.6) to 7(33.3) respectively. This might be due to the inability of the suspects to produce good quality sputum, thereby making some results which were 1+ to change to 2+ and some that were 2+ to change to 3+. In Table 1, it is seen that the age range 20-24 and 40-49 in sample 1, had changes in their positive results. The result in age range 20-24 has shown that it is quite difficult to obtain quality sputum samples, which makes TB diagnosis among adolescents challenging. Indeed, many adolescents with TB are prone to producing smear negative sputum samples (Y. L. J. Byeon., 2007).

Up to 30% of patients may be unable to produce a sputum specimen even under optimal conditions. Collection of good sputum samples depend on thorough health care worker education and patient understanding throughout all phases of the collection process. Quality of specimen can be improved by,

1) Obtaining sputum prior to antibiotic treatment;
2) Rinsing mouth prior to expectoration. Mouth washes or gargles with antibacterial substances should not be used. (") Spada and colleagues showed a I log decrease in the mean concentration of contaminating bacteria in sputum samples from patients immediately following a single mouth wash.(Spada EL, et al., 1989).

From this study, it is probable that, due to lack of homogeneity and other factors inherent in sputum, no single parameter in itself will serve as an adequate means of assessment neither is rejection criteria necessary because it was seen that the wrong quality of a sputum sample (WBC<10/LPF, EPC≥25/LPF), had one AFB positive smear result which would have been rejected if rejection criteria was used. In settings where laboratory staff have the capacity to examine all slides, or in settings where a very high proportion of specimens are AFB positive, rejection of no specimens may be the best approach and I also recommend that early morning sputum samples be preferred for TB diagnosis as they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. Twenty four hour sample should be discouraged as there is more chance of contamination and false results.

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