

SPUTUM CONCENTRATION IMPROVES DIAGNOSIS OF PULMONARY TUBERCULOSIS CASES IN CHILDREN AT A TERTIARY CARE INSTITUTION IN RWANDA

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ABSTRACT

Background: Pulmonary tuberculosis diagnosis by direct sputum smear microscopy is still questionable because of its low sensitivity and this technique is not sufficient to diagnose pulmonary tuberculosis especially in people who are not able to spit out properly like children and old people; therefore another more sensitive conventional technique like concentrated sputum smear microscopy is needed in patients suspected of having pulmonary tuberculosis. We aimed to find out if the sputum concentration technique can be more sensitive than direct smear microscopy in the diagnosis of pulmonary tuberculosis according to age groups.

Methods: This study was a cross-sectional, conducted on 70 participants at CHUK. The sputa were examined microscopically on direct and concentrated smears and result compared to culture. Finally the data were analyzed with MS excel and SPSS.

Results: A total of 210 sputa were analyzed by direct and concentration methods with culture as a gold standard. In patients under 15 years both methods were different in sensitivity (25% vs. 75%, CI= 95%, P= 0.047), in patients of 15 years of age and more, both methods had the same sensitivity (75% vs. 75%, CI= 95%, P = 0, 87). Regardless of age groups both methods were different in sensitivity (80% vs.90.9%, C.I= 95%, P = 0.001).

Conclusion: Sputum concentration is more sensitive than direct technique especially in children under 15 years. We would recommend all researchers involved in tuberculosis to increase the sample size and use different study sites to validate this method before its implementation universally.

Keywords: Tuberculosis - Direct microscopy - Sputum concentration - Culture - Sensitivity

RESUME

Introduction : Le diagnostic de la tuberculose pulmonaire par examen microscopique des frottis direct est encore discutable en raison de sa faible sensibilité et ne permet pas elle seule de poser le diagnostic de la tuberculose pulmonaire. Ceci est surtout le cas chez les malades qui ne sont pas en mesure de trouver le crachat à savoir les enfants et les personnes âgées. Par conséquent, une autre technique classique plus sensible comme le frottis concentré est nécessaire dans ce groupe des patients. Ce travail avait pour but de déterminer l'apport réel de la technique de concentration des crachats comparé à celui de l'examen microscopique de frottis direct dans le diagnostic de la tuberculose pulmonaire selon les groupes d'âge.

Méthodes: Il s'agissait d'une étude prospective, transversale portant sur 70 patients suspect de tuberculose pulmonaire vue au CHUK. Les résultats de l'examen microscopique direct; et après concentration du crachat ont été comparés aux résultats de la culture. Les données ont été saisies et analysées avec MS Excel et SPSS.

Résultats: Sur un total de 210 crachats examinés, l'approche d'examen microscopique basées sur la concentration du crachat a détecté un nombre significativement plus élevé chez les patients de moins de 15 ans (25% vs 75%, IC = 95%, P = 0,047). Chez les patients de 15 ans et plus, les deux méthodes ont détectée de façon égale avec une même sensibilité (75% contre 75%, IC = 95%, P = 0, 87). Quel que soit e le groupe d'âge, la méthode d'examen microscopique après concentration du crachat a une sensibilité légèrement supérieur qu' à celle dont l'approche utilise un examen direct (80% vs.90.9%, IC = 95%, P = 0,001).

Conclusion: L'examen microscopique basé sur la concentration du crachat est plus sensible que la technique directe surtout chez les enfants de moins de 15 ans. Nos résultats font appel à des recherches portant sur grande taille de l'échantillon et impliquant différents sites d'étude pour valider cette méthode avant sa mise en œuvre.

Mots-clés: Tuberculose - Microscopie Directe - Concentration du crachat - Culture - Sensitivité

INTRODUCTION

The social and economic burden of tuberculosis (TB) has been the subject of much study, and major efforts are under way to try to achieve its control. It is estimated that 2 billion people are infected with Mycobacterium tuberculosis (Mtb); at least 10 % of these people (200 million) will develop active TB in their lifetime [19, 20, 21]. TB is one of the major public health problems in Rwanda, where its incidence rate is 386, 71 per 100 000 of the population [9]. The microbiological diagnosis of pulmonary TB by sputum smear microscopy plays a key role in routine diagnosis of TB and treatment follow up in Tuberculosis Control Programs in developing countries, however different researches done explained

the concentration of acid-fast bacilli (AFB) in clinical specimens as an important step in the laboratory diagnosis of mycobacterium diseases [18].

The most accomplished TB control programmes in developing countries are reaching a 56 % case detection rate, primarily by direct sputum microscopy which is rapid, inexpensive and highly specific and capable of identifying the most infectious cases of TB, but its sensitivity is limited, particularly in people who are not able to cough up properly like children under 10 years of age and old people [1, 3, 11].

The gold standard for pulmonary TB diagnosis remains culture, but most developing countries still relies on microscopy for acid-fast bacilli (AFB) in sputum smears for the diagnosis of TB because it is simple, inexpensive and provides rapid results. Unfortunately, the technique has a low sensitivity (43-60 %) when compared with that of cultures [12, 13]

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The sensitivity of direct technique is even lower in pediatric and HIV/AIDS patients [4,5]. Previous studies have shown that concentration and liquefaction of sputum significantly improves the sensitivity of direct microscopy in Patients suspected of having pulmonary tuberculosis but few studies have been done in Children and Old people [7,8,14,19].

In this study we performed a prospective evaluation direct and concentrated smear microscopy on three early-morning sputum specimens from patients suspected of having tuberculosis attending Kigali University Teaching Hospital in Rwanda, a resource-poor setting.

METHODS

This study was conducted on consecutive patients suspected of having symptoms of pulmonary TB attending Kigali University Teaching Hospital during the period between July 2010 and June 2011. Verbal and written informed consents were obtained from the participants. They were informed of the main objective of the study and were requested to sign the form if they agreed to participate in the study and were assured of confidentiality of any disclosures. All sputa from patients under tuberculosis therapy were not included in the evaluation because treatment would interfere by damaging mycobacterium structure resulting in negatives results in culture for dead AFB. A total of 210 early morning sputum samples were collected from 70 patients (3 samples for each). The time and irregularity of TB patients prompted us to use a consecutive sampling method where we took all accessible samples during our research data collection.

The patients were isolated outside rooms, they slightly bent the thoracic part and inhaled as much air as they could in order to induce the coughing reflex; and then they forced the sputum to be coughed in the container placed on the mouth. Early morning sputum samples were preferably collected. Sputum containers were provided, and were capped tightly to avoid any leakage and the labeling was done immediately.

All sputa were analyzed macroscopically to evaluate the quality of the samples; the accepted sputum specimen was analyzed. Smears were prepared within the safety cabinet.

Digestion and decontamination procedures were used in processing sputum for examination and culture of sputum specimens. The concentration technique that was used is sodium hypochlorite 5 % overnight sedimentation method. The supernatant was discarded, the sediment mixed with the remaining fluid and smeared onto a labeled slide and then stained with Ziehl Neelsen technique [2]. The slides were examined under oil immersion (x100 objective). Acid-fast bacteria appear fine red rods against a blue background, and non-acid-fast bacteria (and other organisms and cellular materials) appear blue. The negative slides were considered if there are no acid-fast bacilli in 300 fields [15, 17].

Pellets from direct specimens were inoculated on Lowenstein Jensen medium then the cultures were incubated for eight weeks. They were examined every seven days for possible growth. M. tuberculosis appears as brown granule colonies [19].

The percentages and all calculations were determined

with the aid of Microsoft excel and Statistical Package for Social Sciences (SPSS).

The sensitivity and specificity of the direct and concentrated smear microscopy techniques were calculated using culture result as gold standards [21].

RESULTS

As shown in table 1 below, the results of direct sputum smear microscopy and culture according to age groups were in 14 patients under 15 years of age only 1 (1.4%) patient had positive smear, while 13 (18.6%) negative smear, and 4 (5.7%) positive on culture and 10 (13.3%) negative on culture. In 28 patients between 15 and 40 years old only 4 (5.7%) had positive smear, 24 (34.3%) negative smears, 3 (4.3%) positive on culture and 24 (34.3%) negative smears. In 28 patients over 40 years old only 3 (4.3%) had positive smears, 25 (35.7%) negative smears and 3 (4.3%) positive on culture and 24 (34.3%) negative on culture. 2 (2.8%) samples were characterized by non mycobacterial colonies; therefore they were considered as contaminated.

Table 1: Comparison of results between direct smears and culture according to age

Age	Direct technique		Culture		
	Positive	Negative	Positive	Negative	Contaminated
< 15 years	1 (1.4%)	13 (18.6%)	4 (5.7%)	10 (13.3%)	0 (0%)
15 – 40 years	4 (5.7%)	24 (34.3%)	3 (4.3%)	24 (34.3%)	1 (1.4%)
>40 years	3 (4.3%)	25 (35.7%)	3 (4.3%)	24 (34.3%)	1 (1.4%)
Total (%)	8 (11.4)	62 (88.6%)	10 (14.3%)	58 (82.9%)	2 (2.8%)

While the table 2 shows the results of concentrated sputum smear microscopy and culture according to age. In 14 patients under 15 years old only 3 (4.3%) had positive smear, 11 (15.5%) negative smear, 4 (5.7%) positive on culture and 10 (14.3%) negative on culture. In 28 patients between 15 and 40 years old only 4 (5.7%) had positive smear, 24 (34.3%) negative smears, 3 (4.3%) positive on culture and 24 (34.3%) negative smears. In 28 patients over 40 years old only 3 (4.3%) had positive smears, 25 (35.7%) negative smears and 3 (4.3%) positive on culture and 24 (34.3%) negative on culture. 2 (2.8%) samples were contaminated on culture.

Table 2: Comparison of results between concentrated smears and culture according to age

Age	Concentration technique		Culture		
	Positive	Negative	Positive	Negative	Contaminated
< 15 years	3 (4.3%)	11 (15.5%)	4 (5.7%)	10 (14.3%)	0 (0%)
15 – 40 years	4 (5.7%)	24 (34.3%)	3 (4.3%)	24 (34.3%)	1 (1.4%)
>40 years	3 (4.3%)	25 (35.7%)	3 (4.3%)	24 (34.3%)	1 (1.4%)
Total (%)	10 (14.3%)	60 (85.7%)	10 (14.3%)	58 (82.9%)	2 (2.8%)

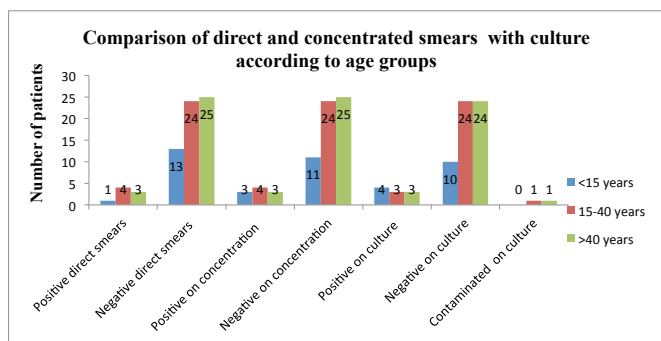


Figure 1: Comparison of direct and concentrated smears with culture

The difference in sensitivity of direct and concentration technique according to age groups referring to culture as gold standard. Among patients aged between 15 and 40 and those aged of more than 40 years, for each group 3 were positive, 24 were negative on culture. In each of these two groups 3 patients were true positive and 1 patient was false positive whereas in patients under 15 years of age both techniques showed different results where on direct technique 1 patient was positive, 13 patients negative, 1 patient was true positive and 3 patients were false negative. But on concentration technique 3 patients were positive, 11 patients were negative and 4 patients were positive on culture thus 3 patients were true positive and 1 patient was true negative (Figure 1).

Sensitivity of direct technique in patients between 15 and 40 years of age = $\frac{3}{3+1} \cdot 100 = 75\%$

Sensitivity of direct technique in patients over 40 years of age = $\frac{3}{3+1} \cdot 100 = 75\%$

Sensitivity of direct technique in patients under 15 years of age = $\frac{1}{1+3} \cdot 100 = 25\%$

Sensitivity of concentration technique in patients under 15 years of age = $\frac{3}{3+1} \cdot 100 = 75\%$

Sensitivity of direct and concentration technique in patients of 15-40 years and more than 40 years of age was not significantly different (75% vs. 75%, 95% of confidence interval, P = 0,87) whereas sensitivity of direct and concentration technique in patients under 15 years of age was significantly different (25% vs. 75%, difference 50%, 95% of confidence interval, P= 0.047). Sixty two patients were tested negative and 8 positive by the direct method, whereas 60 were negative and 10 positive by the concentration method (Figure 2). Referring to the culture as gold standard, on direct method 2 were false negative. On concentration 1 patient was false positive and 1 was false negative on culture.

Sensitivity of direct method = $\frac{8}{8+2} \cdot 100 = 80\%$

Sensitivity of concentration technique = $\frac{10}{10+1} \cdot 100 = 90.9\%$

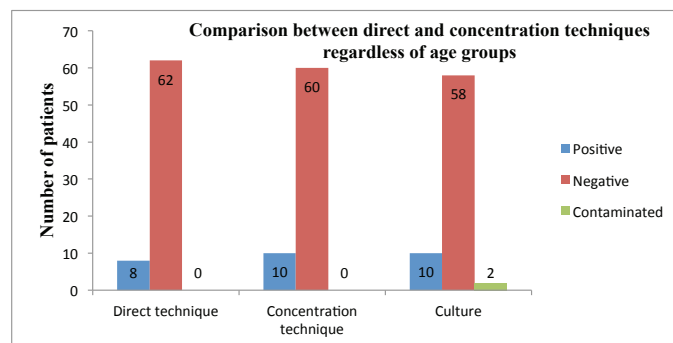


Figure 2: Comparison between direct and concentration techniques

Regardless of age groups the sensitivity of direct and concentration technique was significantly different (90.9% vs.80%, difference =10.9%, C.I= 95%, P = 0.001). This gives strong evidence that concentration method is more sensitive than direct method.

DISCUSSION

Direct microscopy of sputum is still the backbone for diagnosing pulmonary tuberculosis, the study aimed at increasing the sensitivity of tuberculosis diagnosis by concentration after pre-treatment with sodium hypochlorite which also makes sputum samples safe to be handled by laboratory workers.

In patients under 15 years old, sputum concentration technique showed a difference comparing to the direct smear microscopy (75% vs. 25%, C.I = 95%, P < 0.05) this difference is in agreement with the findings found in Kenya [14] where the sensitivity was (26.7%vs 21.7%,CI= 95%, P< 0.05). Our findings are also in agreement with the results found currently in Mindouli Hospital in Republic of Congo [9], where the sensitivity of direct and concentration technique in pediatric age was totally different (47.9%vs 87.5%, 95% CI 6.5-18.6, P = 0.001).

These above results from two studies are similar to our findings because their participants were in same age groups (pediatric age < 15 years) and we used the same concentration method.

Generally regardless of age groups sputum concentration technique is more sensitive than direct (90.9% vs.80%, difference =10.9%, C.I= 95%, P = 0.001). Our findings are in accordance with the study done in India [10] where their results in both methods were (13.02% vs. 23.13%, difference = 7, 11%, CI=95%, P=0.001021). This similarity is explained by the use of the same concentration method (using 5% Sodium hypochlorite) and the smears were read by two observers separately to avoid observer's bias. Our findings are also in accordance with the study done in Ethiopia/Adiss Abbaba [15]. about the difference in sensitivity of direct and concentration technique, their results in both methods were (25%vs 34% , difference = 11% C.I= 95%,P=< 0.001) whereas our results in both method concentration and direct techniques were (90.9% vs.80%, difference =10.9%, C.I= 95%, P = 0.001). This similarity is explained by the use of the same sampling method (consecutive method) where we all used the available patients in the research period and we used also

the same sampling criteria where the inclusion criteria were all sputa collected in early morning and 3 samples were required at the same time and needed to fulfill the requirements of good sputum such as purulent sputum.

CONCLUSION

In conclusion, the sensitivity of the concentration technique was markedly increased in pediatric age (< 15 years), this increase has influenced the overall sensitivity in all patients. Considering the low cost and safety of the technique and greater sensitivity, this method can be of vital importance at least for patients under 15 years of age with negative smears on direct technique.

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