Tropical Medicine and Health Vol. 36 No. 3, 2008, pp. 127-130

Original

Bacteriology of Childhood Tuberculosis in Ibadan, Nigeria: A Five-year review

Kehinde Aderemi Oludiran[1*], Oladokun Regina Eziuka[2], Ige Olusoji Mayowa[3], Bakare Rasheed Ajani[1] and Osinusi Kikelomo[2]

Received 9 May, 2008 Accepted 12 June, 2008 Published 8 August, 2008

Keywords: Childhood tuberculosis, Diagnosis, Nigeria

INTRODUCTION

The World Health Organization (WHO) estimates that 1.3 million cases of tuberculosis (TB) and 450,000 deaths from the disease occur annually among children in developing countries [1]. Nigeria currently has the fifth highest burden of human TB in the world, with an incidence of 311 and a mortality of 81 cases per 100,000 population in 2006 [2]. National Tuberculosis Control Programs (NTCP) in many of the TB endemic countries previously neglected childhood infection as it was thought that children rarely developed sputum smear positive disease and therefore contributed little to disease transmission in the community [1, 3]. In recent times, however, awareness of the severity of TB-related morbidity and mortality among children has increased [4].

Establishing a definitive diagnosis of childhood TB has remained a challenge worldwide. The situation is even more worrisome in resource-limited settings of sub-Saharan Africa and Asia where a significant number of cases occur [2]. Accurate diagnosis and effective treatment of cases is essential to reduce the burden of childhood TB. The effective treatment of cases has been facilitated by two recent landmark developments.

First, the World Health Organization (WHO) recently published guidelines for NTCP on the management of TB in children, and secondly the Global Drug Facility, an affiliate of WHO, has made child-friendly drug formulations available in endemic countries [5].

These two positive developments, however, do not address the tenacious problems of diagnosing TB in children, particularly in TB endemic settings with limited resources [5].

The contribution of diagnostic bacteriology in the control of childhood TB in many of the high burden countries of sub-Saharan Africa is largely unknown [6]. Furthermore, data on laboratory diagnosis of childhood TB in Nigeria is not readily available [7] in spite of the high ranking among the WHO-designated countries with the highest burden of the disease [2]. This study was therefore carried out to determine the yield for *Mycobacterium tuberculosis* in specimens from children suspected to have TB in Ibadan.

MATERIALS AND METHODS

This five year (June 2003- May 2007) retrospective study was carried out at University College Hospital (UCH), Ibadan, Nigeria. The TB laboratory in the UCH is a regional laboratory for diagnosis of TB in Southwestern Nigeria. It receives support from Damien Foundation, Belgium through the NTCP.

Specimens were collected from UCH and other adjoining health-care facilities. Sputum samples were collected from older children while gastric aspiration was performed in young children unable to expectorate sputum.

Specimens were encoded and transported daily to the laboratory for analysis. The specimen from each newly diagnosed patient had been collected in a well-labeled plastic container covered with a lid and then transported to the laboratory for processing within 24 hours of collection. A direct smear was made from each specimen and stained with Ziehl- Neelson (ZN) reagents (BDH Chemicals Ltd

[1]Department of Medical Microbiology and Parasitology,
[2]Department of Paedriatrics,
[3]Department of Medicine,
College of Medicine, University of Ibadan, Nigeria
* **Corresponding Author:** Dr AO Kehinde, TB Unit, Department of Medical Microbiology & Parasitology, College of Medicine, University of Ibadan, University College Hospital, Ibadan, Nigeria
Email: aokehinde@yahoo.com

Poole, England) using a known acid-fast bacilli (AFB) stained slide as positive control and a stained slide made of egg albumin as negative control. Quality control of the Z-N reagents was included with every staining slide. Results were recorded according to the grading system of the International Union Against Tuberculosis and Lung Diseases (IUATLD) [8] as -, scanty, +, ++, or +++ AFB. Each specimen was then decontaminated with 4% NAOH. Concentration was done by spinning in cold centrifuge at 3,000 revolutions per minute for five minutes.

The sediment was cultured onto the Lowenstein-Jensen (LJ) slope (Biomark laboratories, Pune, India) and incubated at 37 ℃ for eight weeks. *Mycobacterium tuberculosis* strain H37RV and sterile LJ medium were used as positive and negative controls respectively. Suspicious isolates on LJ medium were confirmed as ***M. tuberculosis*** by re-staining with Z-N reagents at two, four, six and eight weeks of incubation and by biochemical methods [9].The specimens processed included respiratory (sputum and gastric washings), lymph node aspirates and ascitic fluid. In the case of respiratory specimens, three labeled (a, b and c) samples were collected from each child while only one was collected for lymph node aspirate and ascitic fluid. Specimens collected from children undergoing follow-up for treatment during the study period were not included.

Contamination on LJ medium was determined by looking for visible growth before two weeks of incubation and by carrying out Z-N reaction and biochemical tests [9] on growth after two weeks. Genetic and drug resistance profiling of isolates could not be carried out because of a lack of facilities.

Information on children aged 10 years or less with suspected TB was retrieved from the laboratory records. Demographic data and results of smear microscopy for AFB and culture were collated.

Ethical Approval: Ethical approval was not required because it was a retrospective laboratory-based study.

STATISTICAL ANALYSIS

All data were coded and analyzed using the statistical software SPSS version 10.0 (SPSS Inc, Chicago, IL).

The laboratory variables and demographic characteristics of the patients were described in the form of proportions and percentages. Chi square and Fisher's exact tests (where necessary) were used to measure the association between categorical variables.

RESULTS

Six hundred and thirty specimens were analyzed during the five-year period of the study.

Sputum accounted for 302 (47.9%) while gastric washings, ascitic fluid and aspirates from lymph nodes accounted for 24.1%, 11.7% and 16.3% respectively.

Of the total specimens processed, 352 (55.9%) were from males and 278 (44.1%) from females giving a male to female ratio of 1.27: 1.00.

Concerning the age distribution of the subjects, 41 (6.5%) of the specimens were collected from children < one year of age, 193 (30.6%) from the one - five year age group, while 396 (62.9%) were from children aged six to ten years.

Fifty-six (8.9%) of the total specimens processed were smear positive for AFB while 26 (4.1%) were positive for culture. Overall detection of *M. tuberculosis* was 70 (11.1%), as 14 (2.2%) of the specimens were positive for both microscopy and culture.

Furthermore, using culture diagnosis as a gold standard, the overall sensitivity with smear microscopy was 53.9% while specificity was 97.9%.

More samples were collected and processed during the study period mainly for treatment follow-up but were excluded from the data because the patients were not newly diagnosed.

Concerning the type of specimen processed and the yield, Table 1 shows that 20 (6.6%) of the smear positive specimens and 12 (4.0%) of the culture positive isolates were from sputum, while 36 (11.0%) of the smear positive and 14 (4.3%) of the culture positive isolates were from other specimens (gastric washings, ascitic fluid and lymph

Table 1: Yield of *M. tuberculosis* by specimen

Specimen	Smear microscopy +Ve (%)	Culture +Ve (%)
Sputum (n=302)	20 (6.6%)	12 (4.0%)
Gastric washings (n=152)	16 (10.5%)	6 (3.9%)
Ascitic fluid (n=74)	8 (10.8%)	2 (2.7%)
Lymph node aspirates (n=102)	12 (11.8%)	6 (5.9%)
Total (n= 630)	56 (8.9%)	26 (4.1%)

Smear microscopy: (χ^2=3.80, df = 3, p=0.28) Culture: (χ^2=1.20, df=3, p=0.75)

Table 2: Yield of *M. tuberculosis* by age

Age in years	Smear microscopy +Ve (%)	Culture +Ve (%)
<1 yr (n=41)	4 (9.8%)	1 (2.4%)
1-5 years (n=193)	13 (6.7%)	6 (3.1%)
5-10 years (n=396)	39 (9.8%)	19 (4.8%)
Total (n=630)	56 (8.9%)	26 (4.1%)

Smear microscopy: (χ^2=1.59, df =2, p=0.45) Culture: (χ^2= 1.25, df= 2, p=0.54)

node aspirate). None of the associations between the yield and the types of specimen was statistically significant (p= 0.28 for smear microscopy and p= 0.75 for culture).

Table 2 shows that, of the 56 smear positive specimens, 13 (6.7%) were from the 1-5 year age group while children less than one year of age and 5-10 years of age had an equal percentage (9.8%).

Furthermore, the majority of the 26 culture positive isolates 19, 4.8% were from the 5 - 10 year age group while only one (2.4%) was from a child less than one year of age.

Further analysis revealed no statistical difference in yield among the different age groups (p=0.45 and p= 0.54) for either smear microscopy or culture respectively.
Thirty two (5.1%) of the total specimens cultured were contaminated.

DISCUSSION

The less than 10% yield of *M. tuberculosis* in this study was low. It has been reported that, even under optimal conditions, the organisms can be isolated from fewer than 50% of children with TB [10]. Similarly, in a study on 135 children with clinically suspected pulmonary TB by Salazar et al, *M. tuberculosis* was isolated from only 37% of the subjects [11]. By contrast, up to two-thirds of adults may have smear positive TB [12]. The low yield in children compared to adults has been explained by the different pathophysiology of TB in children. That is, children have closed caseous lesions with a relatively small number of mycobacteria while adults have open cavities which contain large numbers of organisms [13].

A small percentage of the specimens were culture positive (4.1%), which was lower than the yield from microscopy. This is in contrast to the study in Sao Paulo where 70% of the culture positive cases were smear positive [11]. Isolation on culture medium detects 10-100 AFB while direct smears require bacterial concentrations of 5-10,000 organisms per ml for detection [8]. Therefore, it is expected that culture will be more sensitive in detecting the organism than microscopy. The high culture contamination rate obtained in this study together with low specificity of smear microscopy might account for the low yield from the culture method. Organisms other than mycobacteria may demonstrate various degrees of acid fastness. Such organisms include *Rhodococcus* species, *Nocardia* species, *Legionella micdadei*, and the cysts of *Cryptosporidium*, *Isospora*, and other coccidian parasites.

In addition, acid-fast stains cannot distinguish between *M. tuberculosis* and non-tuberculous mycobacterium species.

Isolation of *M. tuberculosis* on pure culture is the definitive method of diagnosing TB in adults and children [14]. Many TB laboratories in disease endemic countries of sub-Saharan Africa may not be suitably equipped to perform quality assured routine culture, indicating a need for improved laboratory proficiency and quality assessment to obtain optimal results. Another major drawback of using culture as a gold standard in the diagnosis of TB is the delay arising from the need to incubate for eight weeks. Newer culture methods that eliminate the diagnostic delay associated with LJ medium are promising and may improve TB diagnosis and contribute to more effective TB control especially in the high burden countries of sub-Saharan Africa [15].

There are also new non-bacteriological means of diagnosis such as the T-cell based assays which measure the interferon-γ released after stimulation by *M. tuberculosis* - specific antigen. Although these assays have been recommended for diagnosis of TB in children in low burden countries in the developed world [16], further research is needed to assess their efficacy in resource-poor countries with a high burden of the disease.

The highest yield was from the 5-10 year age group. This is inconsistent with the findings of Vallejo et al who reported a high yield of 70% among neonates [17]. Our yield also did not vary significantly with the type of specimen.

Other types of specimen had only been tested for recovery of *M. tuberculosis* with varying yields [18]. However, certain specimens have been reported to have a very low yield hence not recommended for TB diagnosis [18]. Sensitivity of acid-fast stains for respiratory samples is approximately 70-89% while that of non-respiratory samples is much lower [18].

The increasing culture positivity by age (Table 1) may be due to waning protective effect of BCG vaccine, which tends to be less protective against TB in adults. The efficacy of BCG vaccine with increasing age requires a well conducted prospective study.

Other factors which may affect the yield of the organism are the extent of the disease and HIV status. The fact that study was retrospective and that these factors were not included in the analysis are limitations in the interpretation of the results. Resistance testing was also not carried out.

In conclusion, the low bacteriologic yield of *M. tuberculosis* from the present study underlines the need for a more sensitive tool that overcome challenges of childhood TB diagnosis in resource limited settings.

ACKNOWLEDGEMENTS

The technical support of Mr Felix A Obaseki, chief

laboratory scientist and Mr Lateef Oyewopo, laboratory assistant at the TB Laboratory, UCH, Ibadan, was appreciated The authors also wish to thank Prof Heather Zar of the department of paediatric pulmonology at the University of Cape Town, Republic of South-Africa for her helpful advice.

REFERENCES

1. World Health Organization. Communicable disease cluster. Global tuberculosis report 2002, Geneva, WHO
2. World Health Organization. Global Tuberculosis Control surveillance, planning and financing. WHO report 2008. Geneva, WHO (WHO/HTM/TB/2008.393
3. Chintu C, Mudenda V, Lucas S, et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet 2002; 360: 985-990
4. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Beyers N. The burden of childhood tuberculosis and the accuracy of routine surveillance data in a high-burden setting. Int J Tuberc Lung Dis. 2006; 10: 259-263
5. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. WHO/HTM/TB/2006.371. Geneva: WHO
6. Marais BJ, Pai M. New approaches and emerging technologies in the diagnosis of childhood tuberculosis, Paedriatric Respiratory Review 2007; 8: 124-133
7. Kehinde AO, Ige OM, Dada-Adegbola HO, Obaseki FA, Ishola OCO. Pulmonary tuberculosis in Ibadan: A ten year review of laboratory reports (1996-2005). Afr J Med & med Sci. 2006, 35: 475-478
8. Enarson DA. Laboratory diagnosis of pulmonary tuberculosis. In: Enarson DA, ed. Management of tuberculosis: a guide for low income countries. 5th Edition Paris, France: IUATLD, 2000: 1-50
9. Cowan and Steel. Acid -Fast Rods. In: Barrow GI, Feltham RKA, Editors. Cowan and Steel's Manual for the identification of Medical Bacteria. Cambridge, UK: Cambridge University Press, 1995: pp 91-93
10. Khan EA, Starke JR. Diagnosis of tuberculosis in children: increased need for improved methods. Emerg Infect Dis. 1995; 1: 115-123
11. Salazar GE, Schmitz TI, Cama R, et al. Pulmonary tuberculosis in children in a developing country. Pediatrics 2001; 108: 448-453
12. Daniel TM. Tuberculosis. In: Isselbacter KJ, Braunwald E, Wison JD, Martin JB, Fauel AS, Kasper DL Eds. Harrison's Principles of Internal Medicine. New York, USA: McGraw Hill InC, 1994: pp 710-717
13. Starke JR. Childhood tuberculosis: treatment strategies and recent advances. Paediatric Respiratory Reviews. 2001; 2: 103- 112
14. Grange JM and Zuma A. The global emergency of Tuberculosis: What is the cause? J Roy Soc for the Promotion of Health. 2002, 122: 78-81
15. Foundation for Innovative Diagnostics (FIND) and Becton and Dickson InC. www.bd.com Combined International efforts to improve rapid tuberculosis diagnosis for HIV positive patients in developing countries. Accessed 03/12/04
16. Mazuree GH, Jereb J, Lobue B et al. Guidelines for using QuantiFERON
TB GOLD test for detecting *Mycobacterium tuberculosis* infection. MMWR Recomm Rep. 2005; 54 (RR-15): 49-55
17. Vallejo JG, Ong LT, Starke JR. Clinical features, diagnosis and treatment of tuberculosis in infants. Pediatrics. 1994; 94: 1-7
18. World Health Organization. Laboratory services in tuberculosis control Part 11: Microscopy. 1998, Geneva, WHO (WHO/TB/98.258)