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Original Article

# Prevalence of Latent Tuberculosis Infection in HIV-1-infected Children on Antiretroviral Therapy in Jos, Nigeria

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## Abstract

**Background:** There are few studies investigating the prevalence of latent tuberculosis infection (LTBI) in HIV-1-infected children on antiretroviral therapy (ART), but no data from Nigeria. This study determined the prevalence of LTBI in HIV-1-infected children on ART in our clinic. Knowing the prevalence and thus the burden of LTBI could help improve HIV care by enabling targeted isoniazid (INH) prophylaxis. **Method:** This observational study was carried out from September 2016 to August 2017 at the pediatric HIV clinic of the Jos University Teaching Hospital among HIV-1-infected children on ART, aged 6 months–15 years. LTBI was diagnosed using an interferon-gamma release assay, the ELISpot test, T-SPOT®.TB assay (Oxford Immunotec, Abingdon, UK) on freshly collected whole blood samples within 2 h. Children with a positive test were treated with INH after first excluding TB by chest X-ray and clinical evaluation. **Results:** Of the 90 children studied, 4 (4.4%) had LTBI diagnosed by ELISpot. Their median interquartile range (IQR) age was 10.4 years (7.9–12.5), the majority were male (54.4%) and most of them had originally received Bacille Calmette-Guérin (83/89, 93.3%). They had a median CD4 count of 694 cells/μL (472–1045). The median (IQR) CD4 count was higher in LTBI compared to non-LTBI children: 1286 cells/μL (953–1375) versus 683 cells/μL (465–1040), ($P = 0.044$). **Conclusion:** Although this study showed a very low prevalence of LTBI in our setting, it was still beneficial to the few children on ART identified with LTBI as it enabled treatment with INH. A larger study will be required to ascertain the actual burden of LTBI in such children in our setting.

**Keywords:** Antiretroviral therapy, children, ELISpot, HIV-1, latent tuberculosis, prevalence

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## Introduction

The prevalence of latent tuberculosis infection (LTBI) in non-HIV-1 infected children as well as antiretroviral therapy (ART) naïve HIV-1-infected children is well reported in several studies.[1-9] These studies cut across low to high tuberculosis (TB) prevalence countries and report the various prevalence of LTBI in the study populations. However, there is a paucity of data on the prevalence of LTBI in HIV-1-infected children receiving ART in sub-Saharan Africa. One study from Botswana using interferon-gamma release assay (IGRA) (Quantiferon, QuantiFERON TB Gold [QFT]) reported LTBI prevalence of 1.0% (1/98) among HIV-positive children on ART.[10] In another study from South Africa among both HIV-infected and HIV-uninfected children, using various tests, the prevalence of LTBI at baseline among the 299 HIV-infected on ART were: 31.5% (93/295) for tuberculin skin test (TST), 21.8% (59/271) for QFT test, and 14.8% (39/263) for ELISpot test.[11] There are no data on the prevalence of LTBI in children on ART in Nigeria. This study determined the prevalence of LTBI in HIV-1-infected children on ART in our local HIV clinic. Knowing the prevalence and thus the burden of LTBI could help improve HIV care by enabling isoniazid (INH) prophylaxis.

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# Method

## Study setting

This study was carried out at the pediatric HIV clinic of the Jos University Teaching Hospital (JUTH), Jos, Nigeria, a clinic supported by the AIDS Prevention Initiative in Nigeria (APIN). APIN also supports the sister adult clinic in the same location. Both clinics provide HIV care services for the city of Jos with a population of about 900,000.[12] The pediatric clinic, which came into existence since 2005, is one of the largest in North-Central Nigeria with a large number of children ART.

## Study design/participants

This was an observational study, in which children receiving ART were consecutively recruited and simultaneously screened for LTBI by TST and IGRA, from September 2016 to August 2017.

Study participants were HIV-1-infected children, aged 6 months–15 years, receiving anti-retroviral therapy.

## Inclusion and exclusion criteria

HIV-positive children on ART, routinely attending the pediatric HIV clinic, aged 6 months–15 years, whose parents gave consent for this study and who had no evidence of active TB disease were included, while those with active TB disease were excluded. Any child with signs and symptoms of TB with a suggestive chest X-ray (CXR), and a positive Gene X-pert/smear microscopy (Confirmed TB) or a negative Gene X-pert/smear microscopy (unconfirmed TB) was considered to have TB disease.[13]

## Definition of terms used in the study

LTBI in a child was defined as the presence of positive ELISpot result with or without a positive TST in the absence of active TB disease. Although ELISpot and IGRA are used here for defining LTBI, there is no gold standard test for the diagnosis of LTBI,[3,14] ELISpot is preferred to TST because it is specific for *Mycobacterium tuberculosis* (MTB) and not confounded by Bacille Calmette-Guérin (BCG) vaccine record. Severe immunodeficiency was defined using the WHO criteria, based on absolute CD4 + cell count and age as follows: <1500/mm$^3$ for children <11 months, <750/mm$^3$ for those 12–35 months, <350/mm$^3$ for those 36–59 months, and <200/mm$^3$ for those >5 years.[15]

## Recruitment and data collection

Children who met the inclusion criteria were consecutively enrolled into the study on each clinic visit day. This was a convenience sample, as reagents were limited. Relevant sociodemographic, clinical, and laboratory data were collected. The data collection form included TB screening questions on symptoms and signs. Children asymptomatic and with no signs of TB were recruited into the study. The most recent CD4 T-cell count and viral load of each child were also recorded. For every child, a blood sample for the ELISpot test (up to 6 ml) was always collected before performing the TST.

## Tuberculin skin testing

A TST, based on the Mantoux method was carried out by the intradermal injection of 0.1 mL of 5 TU purified protein derivative (ARKRAY Healthcare Pvt. Ltd., Mumbai, India) and results read within 48–72 h. A test with an induration >5 mm was considered positive for HIV-positive children, while an induration of <5 mm as negative, in line with WHO recommendations.[16]

## ELISpot test

A commercially available ELISpot test, T-SPOT®.TB assay (Oxford Immunotec, Abingdon, UK) was used and test performed as described in the manufacturer's manual (TSPOT. TB package insert, version 2, 2013). This is an *in vitro* diagnostic test used for detecting effector T cells that respond to stimulation by MTB-specific antigens. The test was performed on freshly collected whole blood samples within 2 h of sample collection.

The interpretation and assay criteria to determine whether a patient sample was "positive" or "negative" was according to the manufacturer's guidance.

## Chest X-ray and management of latent tuberculosis infection

All children with a positive TST or ELISpot or both had a physical examination and CXR done to ascertain that they did not have active TB disease, in addition to the initial symptoms and signs screening for TB, before been commenced on INH prophylaxis.

Children were placed on INH for 6 months with a 2-monthly follow-up during this period. Clinical monitoring, including weight measurements were carried out during follow-up.

## Statistical analysis

Descriptive statistics were used to determine the prevalence of LTBI and the proportion of LTBI cases using ELISpot and TST. Wilcoxon-Mann–Whitney test (Wilcoxon rank-sum test) and Chi-square or Fisher's exact were used for comparing nonnormally distributed and categorical variables, respectively, between those with LTBI and those without LTBI. The detection rates for LTBI using ELISpot and TST were compared using a two-sample test, while Kappa statistics were used to determine the agreement (concordance) between the two tests. All analyses were performed using Stata software version 10.0 (Stata Corporation, College Station, Texas, USA), and all tests were two-sided with a $P < 0.05$ considered statistically significant.

## Ethics approval

Ethics approval for this study was obtained from the Ethics Committee of the JUTH, Jos and consent for the study was obtained from the parents/caregivers of the study participants.

# Results

Of the 90 children studied, 4 (4.4%) had LTBI (diagnosed by IGRA). Their median age was 10.4 years (7.9–12.5), the

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majority were males (49, 54.4%) and most of them had received BCG (83/89, 93.3%). They had a median CD4 count of 694 cells/μL (472–1045). Most (77/83, 92.8%) of their mothers were HIV positive and also on ART. The characteristics of the children are shown in Table 1.

Of the 4 children diagnosed with LTBI: 4 (100%) had received BCG according to records, 2 (50%) had an identifiable BCG scar, one (25%) had a history of TB contact, one (25%) was previously treated for TB (more than 1 year ago), and their median CD4 count was 1286 cells/μL (953–1375). None of the 4 LTBI cases had severe immunosuppression. The median interquartile range CD4 count was higher in LTBI compared to ELISpot-negative children: 1286 cells/μL (953–1375) versus 683 cells/μL (465–1040), ($P = 0.044$).

**Table 1: Characteristics of HIV-infected children receiving antiretroviral therapy studied for latent TB infection as defined by a positive ELISpot**

| Characteristics | Total *n* (%) | LTBI (ELISpot) | | *P* |
|---|---|---|---|---|
| | | Present *n* (%) | Absent *n* (%) | |
| Age (years) | | | | 0.792 |
| 0-5 | 5 (5.6) | 0 (0.0) | 5 (5.8) | |
| 6-15 | 85 (94.4) | 4 (100) | 81 (94.2) | |
| Median (IQR) | 10.4 (7.9-12.5) | 9.5 (8.8-11.9) | 10.5 (7.6-12.5) | 0.938 |
| Sex | | | | 1.000 |
| Male | 49 (54.4) | 2 (50.0) | 47 (54.6) | |
| Female | 41 (45.6) | 2 (50.0) | 39 (45.4) | |
| BCG vaccination | | | | 1.000 |
| Yes | 83 (92.2) | 4 (100) | 79 (92.9) | |
| No | 6 (6.7) | 0 (00.0) | 6 (7.1) | |
| Don't know | 1 (1.1) | | | |
| BCG scar | | | | 1.000 |
| Present | 48 (53.3) | 2 (50.0) | 46 (53.5) | |
| Absent | 42 (46.7) | 2 (50.0) | 40 (46.5) | |
| History of TB contact | | | | 0.442 |
| Yes | 7 (7.8) | 1 (25.0) | 6 (7.0) | |
| No | 78 (86.7) | 3 (75.0) | 75 (87.2) | |
| Don't know | 5 (5.5) | 0 (00.0) | 5 (5.8) | |
| Previous TB treatment | | | | 1.000 |
| Yes | 27 (30.0) | 1 (25.0) | 26 (31.3) | |
| No | 60 (66.7) | 3 (75.0) | 57 (68.7) | |
| Don't know | 3 (3.3) | | | |
| Education level of child | | | | 0.592 |
| Pre-school | 6 (6.7) | 0 (00.0) | 6 (7.0) | |
| Pre-primary | 14 (15.5) | 0 (100) | 14 (16.3) | |
| Primary | 52 (57.8) | 4 (00.0) | 48 (55.8) | |
| Secondary | 18 (20.0) | 0 (00.0) | 18 (20.9) | |
| Mother on ART | | | | 1.000 |
| Yes | 77 (85.6) | 4 (100) | 73 (92.4) | |
| No | 6 (6.7) | 0 (00.0) | 5 (7.6) | |
| Don't know | 7 (7.7) | | | |
| Family size | | | | |
| Median (IQR) | 5 (6-7) | 6 (5-8) | 6 (5-7) | 0.803 |
| BMI Z score | | | | 1.000 |
| <-3.0 | 3 (3.3) | 0 (00.0) | 3 (3.5) | |
| >-3.0 | 87 (96.7) | 4 (100) | 83 (96.5) | |
| Median (IQR) | -0.5 (-1.1-0.4) | -0.2 (-0.9-0.7) | -0.5 (-1.1-0.4) | 0.463 |
| Severe immunosuppression* | | | | 1.000 |
| Present | 12 (13.3) | 0 (00.0) | 12 (14.0) | |
| Absent | 78 (86.7) | 4 (100) | 74 (86.0) | |
| CD4 cell count (cells/μL) | | | | |
| Median (IQR) | 694 (472-1045) | 1286 (953-1375) | 683 (465-1040) | 0.044 |
| CD4%, Median (IQR) | 33 (20-42) | 44 (39-48) | 31 (20-42) | 0.060 |
| Viral load (copies/mL) | | | | |
| Median (IQR) | 10 (10-3694) | 10 (10-29) | 10 (10-5200) | 0.260 |

TST=tuberculin skin testing. LTBI=latent tuberculosis infection. *Severe immunodeficiency based on World Health Organisation (WHO) classification.

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Out of the 90 children studied, MTB sensitization was detected by ELISpot in 4/90 (4.4%) children compared to TST in 6/90 (6.7%). Both tests detected sensitization in 3/90 (3.3%) children. There was a moderate agreement between the two tests ($\kappa = 0.58$, $P = 0.0001$).

## Discussion

The prevalence of LBTI in our study population was low (4.4%). This prevalence is closer to the LTBI prevalence of 1.0% (1/98) reported from Botswana using IGRA (Quantiferon, QFT) among HIV-positive children on ART,[10] but lower than the 14.8% (39/263) for ELISpot test reported in South African children on ART.[11]

The low LTBI prevalence in our study could be due to several reasons, one of which is the small sample size studied. Another possible explanation is in the level of CD4 cell counts of our study population which though within normal ranges (694 cells/μL [472–1045]), was not as high as a median value (ranging from 782 to 1941 cells/μL for children ages 17 years down to 1 year) reported in a non-HIV infected, healthy pediatric population in Nigeria.[17] The CD4 cell count is usually a reflection of the presence of effector T- cells that produce interferon-gamma. This is reflected in our study, where children with LTBI had a higher CD4 cell count compared to those without LTBI: (1286 cells/μL (953–1375) versus 683 cells/μL (465–1040), ($P = 0.044$) and this could explain why there was a better immunological response and therefore better detection of LTBI by ELISpot test in the former group.

Again, the low LTBI prevalence in this study may be attributable to our study population's within normal CD4 cell count which is similar to that of the non-HIV infected pediatric population[17] and this would imply that they are at no greater risk of LTBI than the latter since they too will be better able to fight infections such as MTB. However, our study lacked a non-HIV-infected group needed as a control group for comparison in order to ascertain such a claim. It would seem that when CD4 cell counts are within normal in HIV-infected children on ART as in the case of the Botswana study (962 cells/μL [7–2395]),[10] LTBI prevalence appears lower. However, the within normal CD4 count may not be the only explanation for lower LTBI prevalence, as can be seen in the case of a Mexican study using QFT or TST, where the HIV-infected children on ART despite a normal median CD4 count of 913 cells/μL, still had a high LTBI prevalence of 20.3%.[18]

As an alternative explanation, the low LTBI prevalence in our study may be a result of the health education that the children and their parents/caregivers were constantly receiving during their regular 1-monthly or 2-monthly clinic visits. During such visits, the health talks focused on ART adherence, the importance of good nutrition and TB preventive measures, amongst others. The majority of the mothers (92.8%) of children studied were also on ART and receiving the same health education at the adult ART clinic. A study in South Africa showed that health education on TB prevention measures increases good infection control practices,[19] and this could indirectly help reduce the incidence/prevalence of both LBTI and active TB in a population.

We observed that four children were ELISpot positive while 6 were TST positive and 3 of them positive by both tests. This implies that of the four children that were ELISpot positive, one was TST negative. This is not surprising as it is known that unlike infection with MTB, infection with other mycobacteria or previous exposure to BCG vaccination could give a positive TST but not a positive ELISpot.[20] Furthermore, our finding of three children being positive by both tests, giving a moderate agreement between the two tests ($\kappa = 0.58$, $P = 0.0001$) was similar to a study by Kruczak *et al*. which showed that there was an agreement between IGRA (QuantiFERON-TB Gold In-Tube test) and TST in adults.[21]

## Conclusion

Although this study showed very low prevalence of LTBI in our setting to screen for LTBI, it was still beneficial as it enabled targeted treatment with INH. A larger study will be required to ascertain the actual burden of LTBI in HIV-infected children in our setting and also in comparison to HIV-negative age-matched controls.

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### Conflicts of interest

There are no conflicts of interest.

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