High prevalence of drug resistance amongst HIV-exposed and -infected children in a tuberculosis prevention trial

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An emergence of drug-resistant tuberculosis (DR-TB) in settings affected by human immunodeficiency virus (HIV) and tuberculosis (TB) has been observed. We investigated the prevalence of DR-TB in P1041, a multicentred, randomised, double-blind trial which compared the administration of isoniazid (INH) to placebo, in HIV-exposed, non-infected, and-infected African infants in the absence of any documented TB exposure. The prevalence of multidrug-resistant TB (MDR-TB) was 22.2% (95% CI 8.5–45.8) and INH monoresistance 5.6% (95% CI 0.1–27.6) among culture-confirmed cases, with all MDR-TB occurring in a single site. There was no association between INH treatment or placebo group, or between HIV infection status, and DR-TB prevalence. There was a high prevalence of DR-TB among HIV-exposed and -infected children. Surveillance of DR-TB among children in high-burden TB-HIV settings should be routine.

KEY WORDS: tuberculosis; resistance; HIV; children; isoniazid prevention

TUBERCULOSIS (TB) is a major problem in children in settings highly endemic for TB and human immunodeficiency virus (HIV) infection, where the dual epidemics cause significant morbidity and mortality among young children.1–4 Infants born to HIV-infected women have a high risk of exposure to Mycobacterium tuberculosis early in life.5 Isoniazid (INH) preventive therapy (IPT) is a cornerstone of post-exposure TB prophylaxis in HIV-infected and non-infected children.6–9 Drug-resistant TB (DR-TB) in young children usually reflects transmitted (primary) DR organisms, often from a close contact.10 Acquired resistance in young children is unusual, based on the paucibacillary nature of the disease.

P1041 was a multi-centred, Phase II–III randomised, double-blind, placebo-controlled trial comparing primary INH, in the absence of documented TB exposure, to placebo in HIV-exposed non-infected and infected infants. Accrual took place between December 2004 and June 2008 in three South African centres (Chris Hani Baragwanath Hospital, Johannesburg; Tygerberg Children’s Hospital, Cape Town; King Edwards Hospital, Durban) and one site in Botswana (Princess Marina Hospital, Gaborone). Infants born to HIV-infected women were identified through prevention of mother-to-child transmission programmes. Infant HIV infection status was determined through HIV-1 DNA polymerase chain reaction (PCR) testing. Non-HIV-infected infants had negative status confirmed by a second DNA PCR at 24 weeks and a negative HIV enzyme-linked immunosorbent assay at 18 months of age.

Participants were enrolled between days 91 and 120 of life. Eligibility criteria included receipt of bacille Calmette-Guérin vaccine by age 30 days, no previous history of TB in the infant or known exposure to a microbiologically confirmed case of TB or a mother still receiving active anti-tuberculosis treatment at birth, as well as the presence of failure to thrive, recurrent pneumonia, chronic diarrhoea or any immunosuppressive conditions other than HIV.

The primary study endpoints were TB disease or
death in HIV-infected children; and latent TB infection, TB disease or death in HIV-exposed non-infected children within 96 weeks post-randomisation. Secondary study objectives in HIV-infected children included determining whether INH prophylaxis reduced the incidence of TB infection at 96 weeks. A secondary objective in non-HIV-infected children was to determine whether INH prophylaxis improved TB disease-free survival.

A total of 548 HIV-infected and 804 non-infected infants were randomised to daily INH or matching placebo for 96 weeks. The study was prematurely discontinued in March 2008 due to lack of efficacy in prolonging TB disease-free survival in HIV-infected children and TB infection-free survival in HIV-exposed, non-infected children.11 HIV-infected infants also received oral cotrimoxazole prophylaxis and had access to antiretroviral therapy.

Among HIV-infected children, protocol-defined TB or death occurred in 52 (19.0%) in the INH group and 53 (19.3%) in the placebo group (\(P = 0.93\)). Among non-HIV-infected children, there was no difference in the incidence of TB infection free survival between the INH (\(n = 39, 10\%\)) and placebo (\(n = 45, 11\%, P = 0.44\)) groups.

We investigated the prevalence of DR-TB among patients with culture-confirmed TB in P1041 and the association between INH administration and DR. We also describe the association between genotypic and phenotypic markers of DR.

METHODS

Phenotypic mycobacterial drug susceptibility testing (DST) in children was not standard of care in all sites at the time of study implementation, although it was recommended for study participants with culture-confirmed disease. In the present study, all available archived mycobacterial isolates were centrally retrospectively analysed for DR in a laboratory using the BACTEC 460 method (BD, Sparks, MD, USA). Culture-positive children without stored specimens were excluded from this analysis. Phenotypic DST for INH and rifampicin (RMP), a line-probe assay (Genotypic® MTBDRplus, Hain Lifescience, Nehren, Germany), gene sequencing for genetic resistance markers (INH and RMP) and genotyping (spoligotyping)12 were completed. RMP and INH resistance together were defined as multidrug-resistant TB (MDR-TB). The prevalence of DR-TB among culture-confirmed cases was estimated by simple proportions and 95% confidence intervals (CIs) using the adjusted Wald method; 95% CIs for odds ratios (ORs) used the exact method and were estimated using StatXact (Cytel Software, Cambridge, MA, USA, 2005).

This study was approved by all local and relevant international ethics committees (Chris Hani Baragwanath Hospital, Johannesburg; Tygerberg Children’s
Hospital, Cape Town; King Edward Hospital, Durban, Princess Marina Hospital, Botswana); informed consent was obtained for participation in the parent study.

RESULTS

Of 22 culture-confirmed TB cases, 18 underwent DST (four cultures were not available for testing). Five of the 18 isolates showed drug resistance, including one INH-monoresistant and four MDR-TB isolates. Clinical characteristics are shown in Table 1. All MDR-TB cases occurred in the Johannesburg site, which accounted for 65% of both study participants and of total follow-up in the first 96 weeks, and where DST was not routinely available for all participants. Children were treated for TB using standard first-line treatment as per the South African National Tuberculosis Programme, with treatment adjusted if clinically indicated once DST results were available.

One HIV-infected child died prior to initiating DR treatment. In two children with MDR-TB, a phenotypic MDR-TB diagnosis was never available at or during treatment (one was lost to follow-up and the other was well at 2 years follow-up despite first-line treatment).

The overall prevalence of MDR-TB was 22.2% (95% CI 8.5–45.8), and it was 33.3% (95% CI 13.6–61.2) among isolates specifically from the Johannesburg site. There was no statistically significant association between the prevalence of DR and either INH vs. the placebo group, or by HIV infection status (Table 2), but power for detection was low. Household adult TB source cases were identified in three cases at the time of TB diagnosis; none had DST recorded, and available bacteriological data were limited. There was no difference between age at TB diagnosis, sex, type of TB or history of adult TB contact between children with and without DR (data not shown). All isolates with phenotypic DR also had genetic markers of resistance. No isolates classified as susceptible based on phenotypic or line-probe assay had genetic markers of resistance.

DISCUSSION

There was a high prevalence of DR-TB among HIV-exposed and infected children, consistent with previous reports of increasing DR in South African adults and children.10,13,14 Of routine hospital-diagnosed culture-confirmed TB in children in the Western Cape during 2007–2009, 14.0% were INH-resistant and 8.6% were MDR-TB.10,14 The high prevalence of MDR-TB in Soweto was unexpected, and requires verification in larger studies. There was an excellent correlation between phenotypic and genotypic DR.

Two children were treated with four drugs, including ethionamide (ETH), which is usually reserved for second-line therapy in the absence of laboratory confirmation of MDR-TB at diagnosis, but with good clinical outcome. Early detection of disease through

Table 2 Prevalence of drug resistance* by treatment arm and HIV status

<table>
<thead>
<tr>
<th>Drug resistance</th>
<th>All subjects (n = 18)</th>
<th>INH arm (n = 10)</th>
<th>Placebo arm (n = 8)</th>
<th>OR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All resistance</td>
<td>% (95%CI)</td>
<td>% (95%CI)</td>
<td>% (95%CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.8 (12.2–51.2)</td>
<td>20.0 (4.6–52.1)</td>
<td>37.5 (13.5–69.6)</td>
<td>0.42 (0.03–5.32)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>MDR-TB</td>
<td>22.2 (8.5–45.8)</td>
<td>20.0 (4.6–52.1)</td>
<td>25.0 (6.3–59.9)</td>
<td>0.75 (0.04–13.43)</td>
<td>1.00</td>
</tr>
<tr>
<td>INH monoresistance</td>
<td>5.6 (0.1–27.6)</td>
<td>0.0 (0.0–27.8)</td>
<td>12.5 (0.1–49.2)</td>
<td>0.00 (0.00–31.20)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

*Based on 18 of the 22 culture-positive samples that were available for drug susceptibility testing.

HIV = human immunodeficiency virus; INH = isoniazid; OR = odds ratio; CI = confidence interval; MDR-TB = multidrug-resistant tuberculosis.

Table 3 Association between phenotypic and genotypic markers of resistance among children with drug-resistant tuberculosis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic resistance</td>
<td>MDR</td>
<td>MDR</td>
<td>MDR</td>
<td>MDR</td>
<td>INH-monoresistant</td>
</tr>
<tr>
<td>Line-probe assay</td>
<td>MDR</td>
<td>MDR</td>
<td>MDR</td>
<td>MDR</td>
<td>INH-monoresistant</td>
</tr>
<tr>
<td>Genetic markers: inhA</td>
<td>WT</td>
<td>−15 MU-T</td>
<td>−15 MU-T</td>
<td>5 MU-T</td>
<td>−15 MU-T</td>
</tr>
<tr>
<td></td>
<td>315 MU-ACC</td>
<td>315 MU-ACC</td>
<td>315 MU-ACC</td>
<td>315 MU-ACC</td>
<td>315 MU-ACC</td>
</tr>
<tr>
<td></td>
<td>526 MU-GAC</td>
<td>526 MU-TAC</td>
<td>526 MU-TAC</td>
<td>531 MU-TTG</td>
<td>315 MU-ACC</td>
</tr>
<tr>
<td>rpoB</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>Genotype</td>
<td>Beijing</td>
<td>Haarlem</td>
<td>Haarlem</td>
<td>Family 28</td>
<td>Beijing</td>
</tr>
</tbody>
</table>

MDR = multidrug-resistant; INH = isoniazid; WT = wild type.
active screening, coupled with the limited disease severity and the inclusion of high-dose INH and ETH, may have contributed to their favourable outcome. Both were non-HIV-infected. Resolution of uncomplicated TB in children has been described.\(^{15}\)

It is difficult to assess the contribution of INH-resistant organisms to failure of INH prophylaxis\(^{16}\) in the P1041 study. Based on our findings, the high frequency of DR was unlikely to have accounted for the failure of primary INH prophylaxis in P1041, although there was limited statistical power to assess an association. No significant increases in DR-TB have been reported in INH prevention studies among HIV-infected adults.

TB contact investigation should include bacteriological evaluation of the source cases to allow for appropriate management of child contacts. The high prevalence of DR-TB emphasises the importance of microbiological confirmation and routine DST in childhood TB in settings with a high burden of TB and HIV. Rapid and accurate methods for TB DST are urgently needed and should also be implemented in children, given their high accuracy in the presence of culture confirmation.\(^{17}\) Our findings have implications for programmatic use of post-exposure IPT due to the unexpectedly high prevalence of MDR-TB isolates. Routine post-exposure IPT for children exposed to susceptible TB, however, remains critically important.

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References


Dans les contextes où règnent à la fois le virus de l’immunodéficience humaine (VIH) et tuberculose (TB), on voit émerger la TB à germes résistants aux médicaments (TB-DR). Nous avons investigué la prévalence de la TB-DR dans l’essai P1041, un essai multicentrique randomisé et en double aveugle qui comparait l’administration de l’isoniazide (INH) avec le placebo chez les nourrissons africains exposés au VIH et non-infectés ou exposés au VIH et infectés en l’absence de toute exposition connue à la TB. La prévalence de la tuberculose multidrogorésistante (TB-MDR) a été de 22,2% (IC95% 8,5–45,8) et la monorésistance à l’INH de 5,6% (IC95% 0,1–27,6) parmi les cas confirmés par la culture. Tous les cas de TB-MDR sont survenus dans un seul site. Il n’y a pas eu d’association entre la prévalence de la TB-DR et le groupe de traitement INH et le groupe placebo ou entre les statuts d’infection VIH. On note une prévalence élevée de TB-DR parmi les enfants exposés au VIH et infectés par lui. La surveillance de la TB-DR chez les enfants devrait être assurée en routine dans les contextes à fardeau élevé de TB-VIH.

RÉSUMÉ

En los entornos donde se presenta la tuberculosis (TB) y la infección por el virus de la inmunodeficiencia humana (VIH) se observa la aparición de TB resistente (TB-DR). Se investigó la prevalencia de TB-DR en el estudio P1041, un ensayo multicéntrico aleatorizado con doble anonimato, en el cual se comparaba la administración de isoniazida (INH) contra un placebo, en lactantes africanos expuestos al VIH y sin infección, cuando no existía ningún antecedente de exposición a la TB. La prevalencia de TB multidrogorresistente (TB-MDR) fue 22,2% (IC95% 8,5–45,8) y de monorresistencia a INH fue 5,6% (IC95% 0,1–27,6) en los casos confirmados por cultivo y todos los casos de TB-MDR ocurrieron en un solo centro. No se observó ninguna asociación entre el grupo de tratamiento por INH o el grupo que recibió placebo ni entre la situación con respecto a la infección por el VIH y la prevalencia de TB-DR. Existe una alta prevalencia de TB-DR en los niños expuestos al VIH y los niños infectados por el virus. La vigilancia de la TB-DR a los medicamentos debería constituir una práctica corriente en los entornos que presentan una alta tasa de coinfección por TB y el VIH.

RÉSUMEN