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HIGH PREVALENCE OF DRUG RESISTANCE AMONGST HIV-EXPOSED AND INFECTED CHILDREN ON A TUBERCULOSIS PREVENTION TRIAL

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SUMMARY

Background—There is an emergence of drug-resistant-TB (DR-TB) in settings affected by HIV and tuberculosis (TB).

Methods—We investigated the prevalence of DR-TB in P1041, a multi-centred, randomized, double-blind trial which compared administration of INH to placebo, in HIV-exposed-uninfected and HIV-infected African infants in the absence of any documented TB exposure.

Results—The prevalence of MDR-TB was 22.2% (95% CI: 8.5–45.8%) and INH monoresistance 5.6% (95% CI 0.1–27.6%) amongst 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.

Conclusions—There was a high prevalence of DR-TB amongst HIV-exposed and infected children. Surveillance of DR-TB amongst children in high-burden TB/HIV settings should be routine.

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BACKGROUND

Tuberculosis (TB) is a major problem in children in settings highly endemic for TB and human Immunodeficiency Virus (HIV) where the dual epidemics cause significant morbidity and mortality amongst 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 uninfected 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 randomized, double-blind, placebo-controlled trial comparing primary INH in the absence of documented TB exposure, to placebo in HIV-exposed uninfected 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 programs. Infant HIV infection status was determined through HIV-1 DNA polymerase chain reaction testing. HIV-uninfected infants had negative status confirmed by a second DNA PCR at 24 weeks and a negative HIV ELISA at 18 months of age. Participants were enrolled between the 91st and 120th day of life. Eligibility criteria included receipt of BCG 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-TB 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 uninfected children within 96 weeks post-randomization. Secondary study objectives in HIV-infected children included to determine whether INH prophylaxis decreased the incidence of TB infection at 96 weeks. A secondary objective in HIV-uninfected children was to determine whether INH prophylaxis improved TB disease-free survival. 548 HIV- infected and 804 - uninfected infants were randomized to daily isoniazid or matching placebo for 96 weeks. The study was prematurely discontinued in March 2008 because of lack of efficacy in prolonging TB disease-free survival in HIV-infected children and TB infection-free survival in HIV-exposed uninfected children (11). HIV-infected infants also received oral co-trimoxazole 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 HIV-uninfected 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 amongst 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 recommended for study participants with culture-confirmed disease. In the present study, all available archived mycobacterial isolates were centrally retrospectively analyzed for DR in a laboratory using the BACTEC 460 method (Becton Dickinson, MD, USA). Culture-positive children without stored specimens were excluded from this analysis. Phenotypic DST for INH and rifampicin, a line probe assay (GenoType® MTBDRplus, Hain Lifescience, Nehren,

Germany), gene sequencing for genetic resistance markers (INH and rifampicin) and genotyping (spoligotyping)(12) were completed. Rifampicin and INH resistance together were defined as multidrug-resistant (MDR)-TB. The prevalence of DR-TB amongst culture-confirmed cases was estimated by simple proportions and 95% confidence intervals (CI) using the adjusted Wald method; 95% CI for odds ratios used the exact method and were estimated using StatXact (Cytel Software, 2005). This study was approved by all local and relevant international ethics committees; informed consent was obtained for participation in the parent study.

RESULTS

There were 22 total culture-confirmed TB cases, among which 18 had DST undertaken (4 cultures were not available for testing). Five of the 18 isolates showed drug-resistance, including 1 INH monoresistant and 4 MDR. Clinical characteristics are reflected in table 1. All MDR 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 in all participants. Children were treated for TB using standard first-line therapy as per South African National Tuberculosis Program, with treatment adjusted if clinically indicated once DST results were available. An HIV-infected child died prior to initiating DR treatment. In 2 children with MDR-TB, a phenotypic MDR diagnosis was never available at or during treatment (1 was lost to follow-up, the second was well at 2-year follow-up despite treatment with first-line therapy).

The overall prevalence of MDR-TB was 22.2% (95% CI: 8.5–45.8%) and 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 versus the placebo group, or by HIV infection status (table 2), but power for detection was low. Household adult TB source cases were identified in 3 cases at the time of TB diagnosis; none had DST recorded and bacteriological data available were limited. There was no difference between the age at TB diagnosis, gender, 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 amongst 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 (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 4 drugs, which included ethionamide, usually reserved for 2nd line therapy in the absence of laboratory confirmation of MDR at diagnosis, but with good clinical outcome. Possibly early detection of disease through active screening coupled with the limited disease severity and the inclusion of high-dose INH and ethionamide, contributed to their favourable outcome. Both were HIV-uninfected. 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 P1041. 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 amongst HIV-infected adults.

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

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Table 1

Clinical and laboratory characteristics of HIV-exposed and infected children with drug resistant tuberculosis

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
DR	MDR	MDR	MDR	MDR	INH monoresistant
Study arm	Placebo	Placebo	INH	INH	Placebo
HIV status	Negative	Negative	Negative	Positive	Negative
Age at TB diagnosis (months)	15.1	16.4	22.7	6.5	17.6
Gender	Male	Female	Male	Female	Female
TB contact*	Household contact, smear positive, DST unknown	None documented	None documented	Household contact, bacteriology unknown	Household contact, smear positive, DST unknown
Type TB	Pulmonary	Pulmonary	Pulmonary	Pulmonary	Pulmonary
Chest radiographic findings	Hilar adenopathy and alveolar consolidation	Alveolar consolidation	Hilar adenopathy	Hilar and paratracheal adenopathy and alveolar consolidation,	Hilar adenopathy and alveolar consolidation
Regimen received	RIF, INH, PZA, ETO × 6 months	RIF, INH, PZA, ETO × 6 months	RIF, INH, PZA, ETO × 2 months	RIF, INH, PZA, ETO × 2 months; ART started at age 3 months	RIF, INH, PZA, EMB, OFL × 9 months
Outcome at 6 months	Well, remained well at 2 years post-treatment	Well, remained well at 2 years post-treatment*	Lost to follow-up 2 months following initiation of TB therapy**	Died 2 months after TB diagnosis during a hospital admission to initiate MDR therapy	Well at 6 months, lost to follow-up following completion of therapy

*These contacts were only reported at/after TB diagnosis

**Phenotypic MDR diagnosis never available at or during treatment

DR: drug resistance, MDR: multidrug resistance, RIF: rifampicin, INH: isoniazid, PZA: pyrazinamide, ETO: ethionamide; OFX (ofloxacin)

Regimens presented are the final treatment regimen, as recorded by site.

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Table 2

Prevalence of drug resistance* by treatment arm and HIV status

Prevalence of DR (%)	All subjects (n=18)	INH arm (n= 10)	Placebo arm (n=8)	Odds Ratio (95% CI) p-value	HIV-infected (n=7)	HIV-uninfected (n=11)	Odds Ratio (95% CI) p-value
All resistance (95% CI)	27.8% (12.2–51.2)	20.0% (4.6–52.1)	37.5% (13.5–69.6)	0.42 (0.03–5.32) p=0.76	14.3% (0.5–53.3)	36.4% (15.0–4.8)	0.29 (0.01–4.42) p=0.65
MDR resistance (95% CI)	22.2% (8.5–45.80)	20.0% (4.6–52.1)	25.0% (6.3–59.9)	0.75 (0.04–13.43) P=1.00	14.3% (9.5–53.3)	27.3% (9.2–57.1)	0.44 (0.01–7.63) p=0.97
INH mono resistance (95% CI)	5.6% (0.1–27.6)	0.0% (0.0–27.8)	12.5% (0.1–49.2)	0.00 (0.00–31.20) P=0.89	0.0% (0.0–35.4)	9.1% (0.1–39.9)	0.00 (0.00–61.30) p=1.00

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

DR: drug resistance; INH: isoniazid

Table 3

Association between phenotypic and genotypic markers of resistance amongst children with drug-resistant tuberculosis

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**
Phenotypic resistance	MDR	MDR	MDR	MDR	INH monoresistant
Line probe assay	MDR	MDR	MDR	MDR	INH monoresistant
Genetic markers					
inhA prom	WT	-15 MU-T	-15 MU-T	15 MU-T	-15 MU-T
*kat*G	315 MU-ACC	315 MU-ACC	315 MU-ACC	WT	315 MU-ACC
*rpo*B	526 MU-GAC	526 MU-TAC	526 MU-TAC	531 MU-TTG	WT
Genotype	Beijing	Haarlem	Haarlem	Family 28	Beijing

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