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Isoniazid hair concentrations in children with tuberculosis: a proof of concept study

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SUMMARY

Assessing treatment adherence and quantifying exposure to anti-tuberculosis drugs among children is challenging. We undertook a 'proof of concept' study to assess the drug concentrations of isoniazid (INH) in hair as a therapeutic drug monitoring tool. Children aged <12 years initiated on a thrice-weekly treatment regimen including INH (10 mg/kg) for newly diagnosed tuberculosis were enrolled. INH concentrations in hair were measured using liquid chromatography-tandem mass spectrometry at 1, 2, 4 and 6 months after initiating anti-tuberculosis treatment. We found that INH hair concentrations in all children on thrice-weekly INH were detectable and displayed variability across a dynamic range.

KEY WORDS: tuberculosis; paediatric TB; therapeutic drug monitoring; isoniazid drug concentrations; hair assays

TUBERCULOSIS (TB) is a major cause of morbidity and mortality among children living in resource-limited settings.[1] While inadequate adherence is a major barrier to successful treatment in children, suboptimal drug exposure also contributes to treatment failure and drug resistance.[1–3] Questionnaires and pill counts, routinely used to quantify adherence to anti-tuberculosis treatment, are limited by parental/guardian recollection, the provision of answers deemed socially acceptable, and the low accuracy and reliability of pill counts; furthermore, inter-individual variations in pharmacokinetics are not captured in adherence assessments.[4,5] Given the limitations of commonly used adherence metrics, novel methods of therapeutic drug monitoring (TDM)—where drug levels are measured in a biomatrix—of anti-tuberculosis drugs has been sought.[2,6–8.]

TDM using single-plasma drug levels represents only a small window of exposure, has to be collected at a specific time point to be informative, and has had inconsistent success in predicting outcomes.[2,4] TDM may also not reflect typical adherence patterns if adherence improves transiently prior to visits ('white coat effects').[4] Furthermore, phlebotomy is undesirable in children.[9] While TDM in plasma using multiple samples is important for defining pharmacokinetic (PK) parameters such as absorption, distribution, metabolism and clearance, repeated sampling is invasive and impractical in routine clinical practice. A complementary, alternative, non-invasive method of TDM for children on anti-tuberculosis treatment would provide an important clinical tool for the evaluation of exposure and adherence.

The incorporation of drugs into hair from systemic circulation takes over weeks to months,[10] and the monitoring of drug levels in hair has been used previously in epilepsy and human immunodeficiency virus (HIV) infection.[11,12] Another group has examined the relationship between isoniazid (INH) acetylator phenotype and INH hair levels.[13] Our group has expertise in the development of antiretroviral (ARV) assays in hair and in monitoring hair ARV concentrations in HIV treatment and prevention settings to assess exposure-response relationships.[14–17] We present here, for the first time, a study examining INH concentrations in hair among children initiating anti-tuberculosis treatment.

METHODS

A prospective cohort study of children with TB was established at the Byramjee-Jeejeebhoy Government Medical College - Sassoon General Hospital (BJGMC-SGH) in Pune, India. The eligibility criteria

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Table Characteristics of children aged ⩽12 years with TB

Characteristics	(*n* = 38) *n* (%)
Age, months, median [IQR]	64 [24–90]
0–24	11 (29)
>24–<60	7 (18)
>60	20 (53)
Female	18 (47)
BCG scar	26 (68)
Weight, kg, median [IQR]	15.8 [8–19]
Height, cm, median [IQR]	105 [78–116]
HIV-positive	3 (12)
Residence	
Urban	19 (50)
Peri-urban	14 (37)
Rural	5 (13)
Exposure to a known TB case in the past 2 years	12 (32)
Pulmonary TB	18 (47)
Extra-pulmonary TB	20 (53)
Self-reported adherence of >95%	37 (97)

TB = tuberculosis; IQR = interquartile range; BCG = bacille Calmette-Guérin; HIV = human immunodeficiency virus.

included initiation of first-line anti-tuberculosis treatment following a new clinically or microbiologically confirmed TB diagnosis, age ⩽12 years and known HIV status. According to Government of India guidelines, all children, regardless of HIV status, are placed on a standard 6-month fixed-drug combination regimen of thrice-weekly anti-tuberculosis treatment including INH (10 mg/kg).[9] Information about socio-demographic factors, nutritional status, TB risk factors and TB symptoms at the time of enrolment was collected using structured questionnaires. History of the enrolee's previous TB diagnostic results and treatment with ARVs, if relevant, and anti-tuberculosis drugs were obtained from the parent/guardian or medical record abstraction.

At 1, 2, 4 and 6 months after the initiation of anti-tuberculosis treatment, a small thatch of hair (approximately 20 strands) was cut from the occipital region close to the scalp, as previously described.[13,16] The cut hair was placed in tin foil with a patient study identification label taped over the distal end to mark directionality. The specimen was then sealed inside a plastic bag containing a desiccant, stored at room temperature and shipped to the University of California San Francisco Hair Analytical Laboratory for measurement of INH levels.

Briefly, INH was extracted from cut hair samples via methanol/water solution (v/v, 8/2) containing 1% hydrazine dehydrochloride, followed by evaporation and reconstitution prior to separation by liquid chromatography/tandem mass spectrometry. Extracted sample analysis was performed on a Shimadzu LC-20AD HPLC system (Shimadzu Corporation, Kyoto, Japan) coupled to an Applied Biosystems API 5000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) using positive ionisation. The hair samples were not washed before analysis and the extraction was performed only once, as the extraction solution was consistently found to be free of INH following a single extraction. The assay has been validated over the linear dynamic range of 0.5–100 ng INH/mg of hair utilising 20–30 strands of human hair (∼1–3 mg).

The study was designed to establish the range of hair INH concentrations among children on first-line anti-tuberculosis treatment. The median and interquartile ranges (IQR) of INH concentrations in hair were calculated at different time points (1, 2, 4 and 6 months) on anti-tuberculosis treatment. The coefficient of variation (CV) was calculated to assess range in variation. Univariate and multivariate random effects models were constructed to assess the difference in INH concentrations by time point.

The BJGMC-SGH Institutional Ethics Committee, Pune, India, and the institutional review boards of Johns Hopkins University, Baltimore, MD, USA, approved all study methods and procedures. Participants or their parents/guardians provided written informed consent.

RESULTS

Among the 38 children enrolled, the median age was 5.3 years (IQR 2–7.5): 11 (29%) were aged <2 years, 7 (18%) 2–<5 years and 20 ⩾5 years. Eighteen (47%) were female, 3 (12%) had HIV co-infection. The median weight was 16.8 kg (8–19). Twelve (13%) had a history of TB exposure within 2 years of enrolment; 18 (47%) had pulmonary TB and 20 (53%) had extra-pulmonary TB (EPTB). All except one care giver reported >95% adherence to anti-tuberculosis treatment (Table).

The overall median INH concentration was 8.8 ng/mg (range 4.98–15.2) in hair, with a CV of 0.76. The intra-individual CVs ranged between 0.01 and 0.12. Figure A and B shows INH hair concentrations by months on anti-tuberculosis treatment and the intra-individual variability of INH levels, respectively. INH concentrations were comparable for months on anti-tuberculosis treatment; however we found trends for higher hair levels at 4 months of treatment ($P = 0.08$). The multivariate random effects model adjusted for age, sex and type of TB showed that INH levels at month 4 were significantly higher than at any other month ($P = 0.002$).

DISCUSSION

In this study, we characterised the distribution of INH concentrations in small hair samples of children with active TB whose care givers reported >95% adherence to thrice-weekly first-line anti-tuberculosis treatment. We found that hair sampling was acceptable in our setting and that INH hair concentrations in all children on thrice-weekly INH

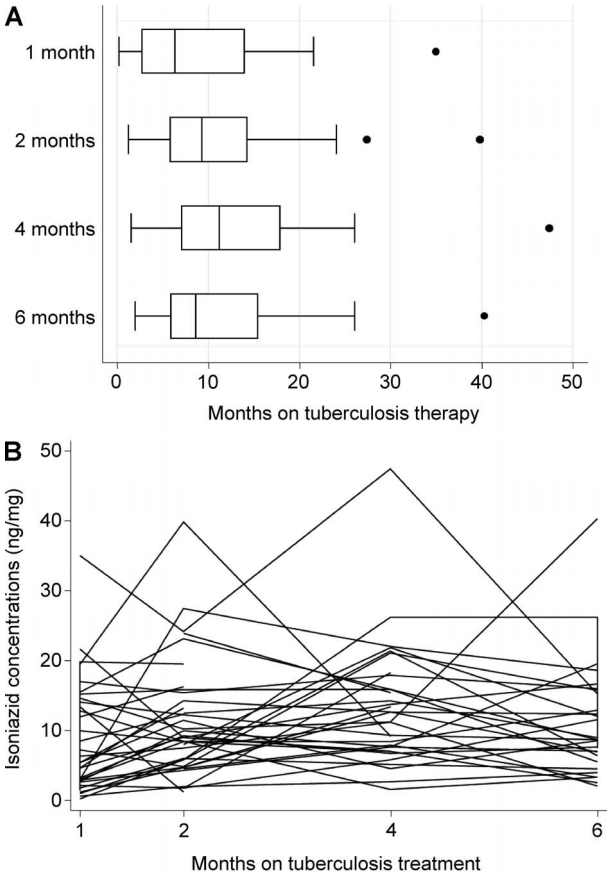

Figure Hair INH concentrations among children at 1, 2, 4 and 6 months on anti-tuberculosis treatment ($n = 38$). **A)** Hair INH concentrations by months on anti-tuberculosis treatment. Months on anti-tuberculosis treatment is shown on the *y*-axis. Dots in the figure represent outlier values. **B)** Spaghetti plot illustrating intra-individual variability of hair INH concentrations over 6 months on anti-tuberculosis treatment. Each line indicates the individual hair INH concentrations at months 1, 2, 4 and 6 of treatment. INH = isoniazid.

were detectable and displayed variability across a dynamic range. In addition, we found that age, sex and duration of exposure to treatment can impact hair concentrations. This study provides 'proof of concept' for using longitudinal measurement of INH in hair as an exposure assessment tool. This innovative TDM method may be useful for evaluating treatment adherence and exposure-response (pharmacokinetic/ pharmacodynamics [PK/PD]) relationships among children on first-line anti-tuberculosis treatment to potentially optimise and individualise drug dosing and reduce adverse events with first- and second-line anti-tuberculosis drugs.

As expected, we found differences in INH hair concentrations among children on anti-tuberculosis treatment by age, sex and duration of exposure to treatment. These differences are likely due to inter- and intra-individual variability in PK values that are dually determined by biology (absorption, distribution, metabolism and clearance of drugs) as well as behaviour (adherence to treatment).[3,6–8] Furthermore, children often display flux in PK parameters due to maturing metabolising systems, making TDM even more important in this population.[9] Although cosmetic hair treatments may influence hair concentrations for certain drugs of abuse, to date we have seen no variability in ARV hair levels based on hair colour or hair treatments in our HIV studies.[13–17] In this study, all of the Indian children had dark hair and none had used hair treatments (colouring, bleaching, straightening, etc.).

Adequate exposure to anti-tuberculosis drugs is essential for achieving optimal treatment outcomes;[9] however, drug administration in children depends on parent/guardian's persistence and children's acceptance of the treatment. TDM using plasma drug levels only estimates a small window of exposure, and also requires skilled phlebotomists, storage and shipment via a cold chain, and sampling timed to dose, which is challenging to achieve in actual practice. Furthermore, some anti-tuberculosis drugs such as INH are unstable in stored plasma samples.[10] Another potential matrix for the TDM of anti-tuberculosis drugs could be dried blood spot (DBS) analysis, as finger prick sampling for DBS preparation is simple. However, this technology is still nascent, the stability of INH in DBS is unknown and DBS assays require standardisation against haemoglobin concentrations and sample volume for interpretation. However, further studies should investigate the complementary use of limited plasma sampling, DBS measurements and hair assays for TDM in the field of TB treatment monitoring.

TDM using hair specimens has several advantages: hair is easy to collect, requires no invasive technique, does not present a biohazard, and can be stored and shipped at ambient temperature. Furthermore, a single measurement in hair approximates exposure over time, similar to area under the concentration time curve measurements from intensive pharmacokinetic studies.[16] This novel method of TDM may have special relevance in the Indian setting, as India has the world's largest TB burden. At present, Indian guidelines recommend only thrice-weekly treatment for children with TB, but this dosing scheme has raised concerns of treatment adequacy,[9] making adherence and the monitoring of exposure even more urgent.

A notable limitation is that hair assays may miss intermittent medication non-adherence. Despite this limitation, this innovative TDM method could have utility in monitoring drug exposure and assessing relationships between longitudinal exposures and treatment response among children on first-line anti-tuberculosis drugs. Further prospective studies are needed to characterise the distribution of INH hair concentrations in different patient populations, evaluate the utility of hair assays of TB drugs in children and adults in predicting TB treatment outcomes, and establish target concentrations of TB

drugs in hair associated with successful treatment outcomes.

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RESUME

Evaluer l'adhésion au traitement et quantifier l'exposition aux médicaments antituberculeux chez les enfants constitue un défi. Nous avons entrepris une étude de « validité de concept » afin d'évaluer les concentrations d'isoniazide (INH) dans les cheveux à titre d'outil de suivi des médicaments. Les enfants âgés de <12 ans, mis sous traitement tri-hebdomadaire comprenant de l'INH (10 mg/kg) pour une tuberculose (TB) récemment diagnostiquée, ont été enrôlés. Les concentrations d'INH dans les cheveux ont été mesurées grâce à la chromatographie liquide couplée à la spectrométrie de masse à 1, 2, 4 et 6 mois après la mise en route du traitement de la TB. Nous avons constaté que les concentrations capillaires d'INH chez tous les enfants sous traitement tri-hebdomadaire étaient détectables et affichaient une variabilité au sein d'une fourchette dynamique.

RESUMEN

La evaluación del cumplimiento terapéutico y la cuantificación de la exposición a los medicamentos antituberculosos en los niños plantean dificultades prácticas. Se llevó a cabo un estudio preliminar de eficacia con el fin de evaluar las concentraciones de isoniazida (INH) en el cabello, como método de seguimiento farmacoterapéutico. Participaron en el estudio niños de edad de <12 años con diagnóstico nuevo de tuberculosis, que iniciaban un tratamiento con una pauta posológica tres veces por semana que comportaba INH (10 mg/kg). Se determinó la concentración de INH en el cabello mediante espectrofotometría de masas acoplada a la cromatografía líquida 1, 2, 4 o 6 meses después de haber iniciado el tratamiento antituberculoso. Se observó que la detección de la concentración de INH en el cabello de todos los niños que recibían INH tres veces por semana era factible y exhibía una variabilidad dentro del intervalo analítico.