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Authors
Mave, V
Chandanwale, A
Kinikar, A
et al.

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Isoniazid Hair Concentrations in Children with Tuberculosis: A Proof of Concept Study

Vidya Mave, MD, MPH, TM[1,2,*], **Ajay Chandanwale, MS**[1,*], **Aarti Kinikar, MD**[1], **Sandhya Khadse, MD**[1], **Anju Kagal, MD**[1], **Nikhil Gupte, PhD**[1,2], **Nishi Suryavanshi, PhD**[1], **Smita Nimkar, MSc**[1], **Hari Koli**[1], **Sultanat Khwaja, MBBS, DCH**[1], **Renu Bharadwaj, MD**[1], **Samir Joshi, MD**[1], **Howard Horng, PhD**[3], **Leslie Z. Benet, PhD**[3], **Geetha Ramachandran, PhD**[4], **Kelly E. Dooley, MD, PhD**[2], **Amita Gupta, MD, MHS**[1,2], and **Monica Gandhi, MD**[3]

[1]Byramjee-Jeejeebhoy Medical College Clinical Trials Unit, Pune, India

[2]Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[3]University of California, San Francisco, USA

[4]National Institute of Research in Tuberculosis, Chennai, India

Abstract

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

Keywords

Tuberculosis; Pediatric TB; therapeutic drug monitoring; Isoniazid drug concentrations; hair assays

Background

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 antitubercular therapy (ATT) adherence are limited by parental/guardian recollection, the provision of

Corresponding author: Vidya Mave, MD, MPH & TM, 1st Floor, Pathology Museum, BJ Medical College Clinical Trials Unit, Jai Prakash Narayan Road, Pune, Maharashtra- 411001, ; Email: vidyamave@gmail.com

*equally contributed to the manuscript

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answers deemed socially acceptable, and the low accuracy and reliability of pill counts; further, inter-individual variations in pharmacokinetics are not captured in adherence assessments [4,5]. Given the limitations of commonly-used metrics of adherence, novel methods of therapeutic drug monitoring (TDM) – where drug levels are measured in a biomatrix– of TB medications has been sought [2,2,6-8].

TDM using single plasma drug levels only represents a small window of exposure, must 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 transiently improves prior to visits ("white coat effects") [4]. Furthermore, in children, phlebotomy is undesirable [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 impracticalin routine clinical practice. A complementary, alternate, noninvasive, method of TDM for children on TB treatment would provide an important clinical tool for the evaluation of exposure and adherence.

Drugs incorporate into hair from the systemic circulation over weeks to months, [10] and the monitoring of drug levels in hair has been utilized previously in epilepsy and HIV infection [11-12]. Another group has examined the relationship between INH acetylator phenotype and INH hair levels[13]. Our group has cultivated expertise in the development of antiretroviral (ARV) assays in hair and 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 isoniazid concentrations in hair among children initiating ATT.

Methods

A prospective cohort study of children with TB was established at BJ Government Medical College (BJGMC) - Sassoon General Hospital (SGH) in Pune, India. The eligibility criteria included initiation of first line ATT following a new clinically or microbiologically confirmed TB diagnosis, age ≤12 years, and known HIV status. Per Government of India guidelines, children regardless of HIV status are placed on a standard fixed-drug combination packets of 6-month regimen of thrice weekly ATT including isoniazid (10 mg/kg) [9]. Information about socio-demographic factors, nutritional status, TB risk factors, and TB symptoms at the time of enrollment was collected via structured questionnaire. History of the enrollee's prior TB diagnostic results and treatment, ARVs, if relevant, and ATT were obtained from the parent/guardian or medical record abstraction.

At 1, 2, 4 and 6 months after ATT initiation, 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 UCSF Hair Analytical Laboratory for measurement of isoniazid levels. Briefly, isoniazid 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 (LC/MS-MS). Extracted sample analysis was performed on a Shimadzu LC-20AD HPLC system coupled to an Applied Biosystem API 5000 triple quadrupole mass spectrometer using positive ionization. The hair samples were not washed prior to analysis and the extraction was performed only once since the extraction solution was found to be free of INH consistently following a single extraction. The assay has been validated over the linear dynamic range of 0.5-100 ng INH/mg of hair utilizing 20-30 strands of human hair (∼1-3 mg).

The study was designed to establish the range of hair isoniazid concentrations among children on first line ATT. The median and interquartile ranges (IQR) of isoniazid concentrations in hair were calculated at different time points (1, 2, 4 and 6 months) on ATT. Coefficient of variation was calculated to assess range in variation. Univariate and multivariate random effects models were constructed to assess the difference in isoniazid concentrations by time point. The BJGMC-SGH Institutional Ethics Committee and Johns Hopkins University institutional review boards both approved all study methods and procedures. Participants or their parents/guardians provided written informed consent.

Results

Of 38 children enrolled, the median age was 5.3 (IQR, 2 - 7.5) years; 11 (29%) were <2 years, 7 (18%) were 2-<5 years and 20 were ≥5 years. Eighteen (47%) were female, 3 (12%) had HIV co-infection. The median weight was 16.8 (8-19) kg and 12 (13%) reported a history of TB exposure within 2 years of enrollment; 18 (47%) had pulmonary TB and 20 (53%) had extrapulmonary TB (EPTB). All except one caregivers reported >95% adherence to ATT (Table).

The overall median isoniazid concentration was 8.8 (4.98 - 15.20) ng/mg in hair with a coefficient of variation (CV) of 0.76. The intra-individual CVs ranged between 0.01-0.12. Figure 1a and 1b depicts isoniazid hair concentrations by months on ATT and the intra-individual variability of isoniazid levels, respectively. Isoniazid concentrations were comparable for months on ATT; however we found trends for higher hair levels at 4 months on ATT ($p = 0.08$). The multivariate random effects model adjusted for age, sex and type of TB showed that isoniazid levels at month 4 were significantly higher than at any other month ($p = 0.002$).

Discussion

In this study, we characterized the distribution of concentrations of isoniazid in small hair samples of children with active TB whose caregivers reported >95% adherence to thrice weekly 1st line TB treatment regimens. We found that hair sampling was acceptable in our setting and that isoniazid hair concentrations in all children on thrice weekly INH were detectable and displayed variability across a dynamic range. In addition, we found that age, gender and duration of treatment exposure can impact hair concentrations. This study provides “proof of concept” for using longitudinal measurement of isoniazid 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 1st line TB drugs to potentially optimize and individualize drug dosing and reduce adverse events to first and second-line anti-TB drugs.

As expected, we found differences in isoniazid hair concentrations among children on TB therapy by age, gender and duration of treatment exposure. These differences are likely due to inter and intra-individual variability in PK that are dually determined by biology (absorption, distribution, metabolism, and clearance of drugs) as well as behavior (adherence to treatment) [3,6-8]. Furthermore, children often display flux in PK parameters due to maturing metabolizing systems, making TDM even more important in this population [9]. Although cosmetic hair treatments may influence hair concentrations for certain drugs of abuse, we have not seen variability in antiretroviral hair levels based on color of the hair or hair treatments in our HIV studies to date [13-17]. In this study, all of the Indian children had dark hair and none of them had used hair treatments (coloring, bleaching, straightening, etc.).

Adequate drug exposure to TB therapy 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 TB drugs such as isoniazid are unstable in stored plasma samples [10].Anotherpotential matrix for TDM of anti-TB drugs could be dried blood spot (DBS) analysis, since fingerprick sampling for DBS preparation is simple. However, this technology is still nascent, the stability of INH in DBS is unknown, and DBS assays require standardization against hemoglobin 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, since hair is simple and noninvasive to collect, does not present a biohazard, and can be stored and shipped at ambient temperature. Further, 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 since India has the world's largest TB burden. At present, Indian guidelines recommend only thrice-weekly therapy for children with TB, but this dosing scheme has raised concerns of treatment adequacy[9], making adherence and exposure monitoring even more urgent.

A notable limitation is that hair assays may miss intermittent medication nonadherence. Despite this limitation, this innovative TDM method could have utility in monitoring drug exposures and assessing relationships between longitudinal exposures and treatment response among children on 1st line TB drugs. Further prospective studies are needed to characterize the distribution of isoniazid 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 to establish target concentrations of TB drugs in hair associated with successful treatment outcomes.

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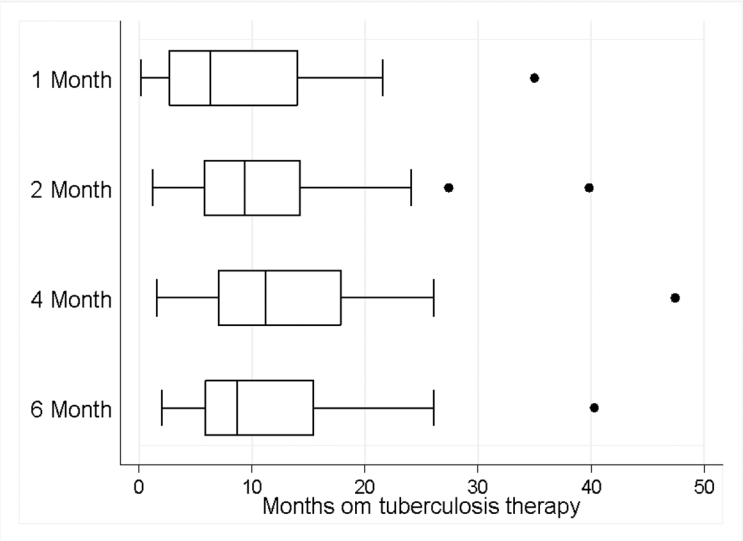

Figure 1a

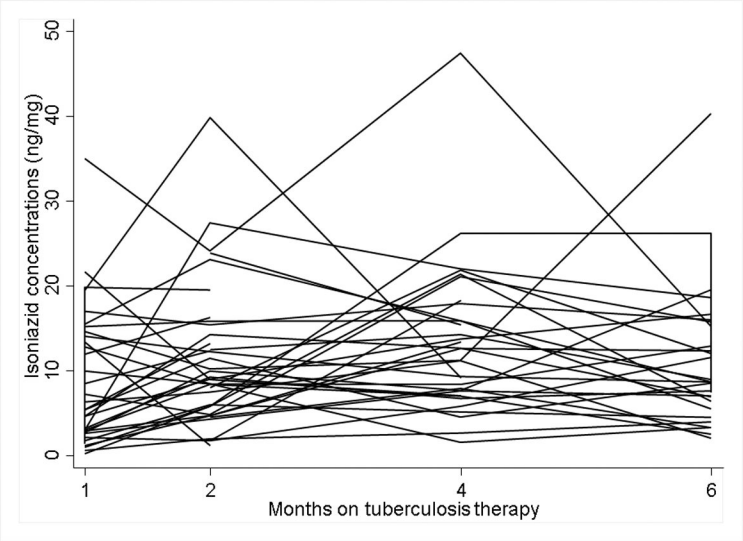

Figure 1b

Figure 1. Hair isoniazid concentrations among children at 1, 2, 4 and 6 months on tuberculosis therapy (n=38)

1a. Hair isoniazid concentrations by months on tuberculosis therapy. Months on tuberculosis therapy is shown in Y-axis. Dots in the figure represent outlier values;

1b. Sphagetti plot illustrating intra-individual variability of hair isoniazid concentrations over 6 months on tuberculosis therapy. Each line indicates the individual hair isoniazid concentrations at month 1, 2, 4 and 6 on tuberculosis therapy.

Table

Characteristic of Children ≤12years of age with Tuberculosis

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

IQR, interquartile range; TB, tuberculosis