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Therapeutic Drug Monitoring of Isoniazid Among Newly Diagnosed Pulmonary Tuberculosis Patients

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ABSTRACT

Introduction: Tuberculosis has a higher incidence in lower income countries. Positive treatment outcomes may be fewer due to comorbidities like diabetes mellitus and immunosuppressive illnesses. The Global Tuberculosis Network working under the umbrella of World Health Organization has composed different committees. The TB Pharmacology Committee has given the concept of precision medicine and treatment based on drug susceptibility testing. Achievement of optimum plasma levels of anti-tuberculous drugs by therapeutic drug monitoring (TDM) is imperative and being emphasized to achieve a TB cure.

Aims and Objectives: To determine the levels of INH in low responders to ATT have lower plasma levels of Isoniazid.

Place and Duration of study: University of Health Sciences and Gulab Devi Chest Hospital, Lahore, for 1 year from August 2017- July 2018.

Material and Methods: A first dose therapeutic drug monitoring (TDM) of isoniazid (INH) was planned in 25 newly enrolled sputum positive tuberculous patients at Gulab-Devi Hospital. The work was approved by Ethical Review Committee. Fixed dose combination (FDC) of anti-tuberculous drugs was given under direct observation and blood samples were withdrawn at two hours (*C2h*) and six hours (*C6h*) on days one, 14 and 56th of drug therapy. Samples of sputum for acid fast bacilli (AFB) were also taken during blood sampling. Method development and validation of isoniazid estimation by high-performance liquid chromatography was carried out. Plasma INH concentration in test samples was measured with Shimadzu Chromatographic System, Japan. Data was entered and analyzed using SPSS version 20. A p value ≤ 0.05 was taken as statistically significant.

Results: Among 25 patients enrolled to the current study, the mean plasma levels of isoniazid were 1.29±0.79 µg / ml and 0.56±0.43 µg / ml at two hours and six hours respectively throughout the research duration. Most of the patients had lesser plasma INH levels than the target ranges (< 3µg / ml). Sputum for acid fast bacilli was found 100% positive on day one and 14 however sputum conversion was 56% after four weeks drug therapy.

Conclusion: An early TDM monitoring has revealed low plasma INH concentration. Correction of dose to achieve expected plasma INH level will have promising effect on sputum culture conversion. It will minimize the total statewide burden of slow responders and tuberculosis resistant cases.

Key Words: Therapeutic drug monitoring (TDM); isoniazid; HPLC.

INTRODUCTION

Tuberculosis (TB) has higher incidence in lower income countries. But comorbidities like HIV and diabetes mellitus make sporadic cases of TB in countries having sound health care systems[1]. Sputum for acid fast bacilli estimation, a cost-effective tool for rapid diagnosis and three to four drug therapy for 06-24 months has been practiced to cure tuberculous disease[2]. An expected cure rate has been dependent, upon the site of infection, physical nature, and sensitivity of organism to the drugs. Treatment outcomes may be meagre due to comorbidities like diabetes mellitus and immunosuppressive illnesses[3]. Eight nations share two thirds of the world's tuberculosis cases, with Pakistan bearing 5.8% of the disease's burden[4]. Global Tuberculosis Network (GTN) working under the umbrella of World Health Organization (WHO) has composed different committees. TB Pharmacology committee has given the concept of precision medicine and treatment based on drug susceptibility testing (DST)[5]. Successful outcomes in tuberculous patients are dependent upon four

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pillars: Pathogen, host, selection of drugs and pharmacokinetic assays of drugs in the recipients. Plasma levels of an antituberculosis drugs less than the expected range have gained little importance because TB patients belong to source limited countries. Routine monitoring of peak plasma drug levels is impracticable and very costly[6,7]. Achievement and maintenance of optimum plasma levels of anti-tuberculous drugs by therapeutic drug monitoring (TDM) are being emphasized[8]. Factors such as genetic polymorphisms unique to Pakistani demographics, concurrent medications commonly used in the region, and prevalent disease states such as malnutrition or comorbidities such as diabetes can all contribute to this variability, emphasizing the importance of personalized dosing strategies. Furthermore, changes in dosage formulations and administration routes which are affected by local pharmacy practices, might affect drug absorption, distribution, metabolism, and excretion. Insufficient data is available on plasma levels of anti-tuberculous drugs in our settings. The aim of the study was to investigate optimum plasma concentration of INH in tuberculous patients and take into consideration the potential factors contributing to the drugs resistance in our community.

MATERIAL AND METHODS

Patients:

This descriptive study was conducted between August 2017 to July 2018 in Pharmacology Department, University of Health Sciences (UHS) and Gulab Devi Chest Hospital, Lahore, Pakistan. The research protocol was approved by UHS Ethical Committee September 2016 (ERC Letter dated 8-09-16). A sample size of 25 was set for this pharmacokinetic study[10]. Newly diagnosed sputum acid fast bacilli (AFB) positive, pulmonary tuberculous patients admitted in hospital were enrolled. Twenty-five patients both males and nonpregnant females within the age limit of ≥18 to 65 years and mycobacterium tuberculosis (MTB) sensitive to 1st line anti-mycobacterial drugs were enrolled. Written, informed consent was taken. Patients < 18 years and having debilitating co-morbidities were excluded from present study. FDC containing isoniazid (300mg), rifampicin (450 or 600 mg) pyrazinamide (maximum 1500 mg) and ethambutol (maximum 850 mg) being practiced in Gulab Devi Chest Hospital, Lahore was prescribed to the test patients. Standardized meals and DOT regimens of the hospital were strictly followed for 8 weeks.

Sample Collection:

Drug was administered with water after overnight fasting. First blood sample (3 ml) was withdrawn in ethylenediamine-acetate (EDTA) coated tubes two hours (*C2h*) after drug intake. Patients were served with standard meal being arranged by the hospital. Second blood sample was taken after 6 hours (*C6h*) post dose on day one, 14 & 56. Samples were centrifuged and serum was collected. The serum was labeled and stored at -80 °C till further processing[11].

Chemicals and Reagents:

Anti-tuberculous drugs were issued from Gulab Devi Hospital Pharmacy: (Batch no: A606195). The fixed dosage combination (FDC) technique already used at Gulab Devi Hospital Lahore was adopted. All the reagents were of analytical grade. Isoniazid (purity 98.00%, w/w), acetonitrile, methanol (HPLC grade), and orthophosphoric acid were purchased from Merck Ltd Germany and Disodium hydrogen phosphate (Na_2HPO_4), from Allied Signal Chemicals, NJ. Double distilled water was prepared at the Quality Operation Laboratory UVAS, Lahore. All other chemicals and reagents were of analytical grade.

Drug Concentration Analysis:

Shimadzu (HPLC) Chromatographic System Japan equipped with a LC-20AT VP pump, an SIL-20AC HT auto sampler, SPD-M20A, CTO 20 AC and CBM 20A controller unit was used. Column C18 (250 x 4.6 mm, particle size 5µm) Merck, Germany was selected for isoniazid. Mobile phases A (95:5 v/v) and B (50:50 v/v) were made with the combination of disodium hydrogen phosphate buffer 0.01M and acetonitrile respectively. Mobile phases were pumped at flow rate of 1ml/min. Wavelength of 238 nm was adjusted on UV detectors. The limits of detection and quantification for isoniazid were 0.3 and one µg/mL respectively[10].

Statistical Analysis:

Statistical Package for Social Sciences (version 20) was used. Mean ± SD was given for quantitative clinical and laboratory parameters. Independent sample t test was used to observe the mean difference in serum concentration between two and six hours. Chi-square/Fisher's exact test was used to adjust the association of qualitative clinical parameters with days. The mean differences in laboratory parameter and serum concentration amongst day one, 14 and day 56 were determined by ANOVA test. A *p*-value ≤0.05 was taken as statistically significant.

RESULTS

A total of 25 newly diagnosed patients with active pulmonary tuberculosis were enrolled. All the subjects were inpatients who received FDC being issued from the hospital pharmacy. Amongst 25 patients, the ratio of male, female was 68% to 32 % respectively; 24% had positive family history. There was improvement in clinical symptoms of cough, night sweats, fever and weight gain and hemoptysis after drug therapy (P-value<0.0001) except for hemoptysis (P-value > 0.120).

Post Dose Blood Sampling		Mean µg/ml	Std. Deviation	Std. Error	Min µg/ml	Max µg/ml	P-value
2 hours	Day 1	1.490	0.865	0.173	0.29	4.93	0.028*
	Day 14	1.394	0.917	0.183	0.36	4.91	
	Day 56	1.004	0.474	0.095	0.02	1.61	
	Aggre-gate mean	1.296	0.796	0.092	0.02	4.93	
6 hours	Day 1	0.733	0.333	0.067	-0.07	1.33	0.002*
	Day 14	0.566	0.484	0.097	-0.19	1.85	
	Day 56	0.490	0.409	0.082	-0.14	1.18	
	Aggre gate mean	0.563	0.432	0.049	-0.19	1.85	

Table-1: Concentrations of isoniazid at *C2hr* and *C6hr* hours in pulmonary tuberculous patients.

Where: Serum INH values are expressed as µg/ml.
*Significant *p*-value < 0.05

Hepatic and renal function tests were assessed during this work. No renal or hepatic toxicity was observed[11].

HPLC method validation of INH showed 95.95% mean recovery at quality checkpoint. High selectivity was observed for INH. There was no interference in the INH peaks by other antituberculosis drugs present in FDC[10].

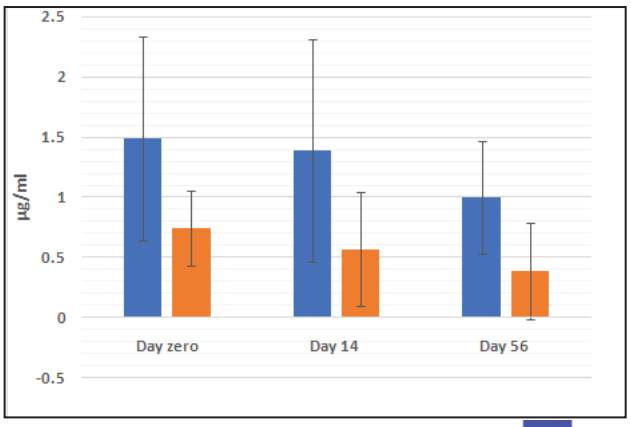

Fig-1: Plasma and isoniazid levels at C_2hr and C_6hr in Pulmonary tuberculous patients taking fixed dose combination of antituberculosis drugs.

Where: n=25 Mean ± SD (µg/ml). * *p*-value <0.05 is significant.

Plasma *C2hr* levels of isoniazid after drug administration on day one was (mean ± SD) 1.49±0.87 µg/ml ranging from 0.29 to 4.93 µg/ml. On day 14 was 1.4±0.92 µg/ml ranging from 0.36 to 4.91µg/ml. After 8 weeks of drug therapy the plasma INH levels at *C2h* were decreased to 1.00±0.47 µg/ml ranging from 0.02 to 1.61µg/ml. There was a significant difference among the plasma levels *p*-value 0.028 (Table-1).

Plasma *C6hr* levels of INH on day one and 14 were 0.734 ±0.334 µg/ml and 0.5656 ±0.487 µg/ml respectively. After 8weeks *C6hr* was greatly reduced to 0.490±0.410 µg/ml (Table-1, Fig-1). Positive sputum for AFB was found 100% on day one and 14 but 11 (44%) patients were sputum positive on 56th day. We observed 56% sputum conversion rate after 8 weeks in this study (Table-2, Sr no 1).

Sr. No	Blood Sample	Subjects (n)	*C2hr* Mean ±SD µg/ml [Range]
1	Day 1	n=25	1.49±0.87 [0.29-4.93]
	Day 14	n=25	1.4±0.92 [0.36-4.91]
	8 Weeks	n=25	1.00±0.47 [0.02-1.61]
*2	8 Weeks	*n=28[17]	1-2.6 [0.4-3.3]
*3	8 Weeks	*n=181[18]	1.3 [0.9-2.2]
*4	Day 14	*n=16 [9]	1.85 ± 1.3
*5	Day 14	*n=08[19]	3.1 ± 1.1
*6	Day 7	*n=57[16]	2.5 [1.5-4.3]

Table-2: Comparison of plasma *C2hr* levels of isoniazid reported in tuberculous patients treated with FDC ,*Sr. No 2-6(References)

DISCUSSION

In this study we observed the plasma INH levels at *C2hr* and *C6hr* in 25 adult pulmonary tuberculous patients. The patients were served FDC and were closely supervised for 8 weeks. The distribution of *C2hr* plasma INH was found to be lower than the mean values. There was improvement in clinical symptoms of cough, night sweats, fever and weight gain and hemoptysis after drug therapy (P-value<0.0001)[11]. The cumulative INH level on day one,14 and 56th day was (mean ± SD) 1.296 ± 0.796 µg/ml. There was a significant decline in plasma INH levels P = 0.028 (Table-1). During this day two hours study period 64 % (16) among 25 patients had less than the mean INH levels. The imminent effect of low plasma INH levels was found as 56 % sputum conversion after 8 weeks of drug therapy (Table-2). WHO has reported 87% treatment success rates among the 2.6 million patients in 2011[3]. There are multiple studies showing ≥ 90 % recovery after 2 months intensive care. But we have observed a low sputum conversion rate during this period. Old age, male gender, and bacillary load of 3+ in sputum smears are attributed to delayed conversion[13]. Akhter et al. (2019) reported 63% Pakistani population has shown fast N-acetyltransferase-2 acetylation of dapsone[14]. The association between low plasma concentration and treatment outcome are in line with previous studies [9,15]. Low INH levels cannot be predicted in an individual because in addition to fast acetylation, it may be affected by an inaccurate dose calculation, variable dosage form, malabsorption of drug, low albumin, variable metabolism patterns or and additional drug interactions with FDC. Measurements of plasma INH at single time *C2hr* looks most appropriate and most of the workers have reported plasma INH measurements at *C2hr.*Low *C2hr* plasma INH would have been due to slow absorption of drug and delayed peak plasm concentration of INH. To cater for this delayed peak plasma concentrations, we decided to measure plasma INH level at *C6hr.* The plasma INH levels were very low, in decimals. The mean INH at *C6hr* was 0.5631± 0.432 µg/ml. (Table-1, Fig-1). Fahimi et al (2013) reported 6 hours post dose of INH 1.5 [0.8–2.1] µg/ml[16]. In a latest 2023 study from South Africa low plasma INH levels were reported as (<3µg/ ml)[28] concurring with other studies reported by us in comparison to our findings (Table-2,Sr.No2-6)[9,16-19].Our findings are comparable to observations reported in other studies across the world with a varying rate of delayed sputum conversion. Acquah et al [20] observed 91.9% sputum conversion in Ghana while 91% conversion was in India[20,21] Similarly, sputum smear conversions by Djouma et al. 2015[22] (92%), Mota et al. 2012[23] (74.6%), Lee et al. 2014[24], (78%), Sari et al. 2019[25] (61.5%) and Burhan et al. 2013[18] reported (49%) sputum smear conversions. This data shows that at the end of intensive phase, good recovery in clinical symptoms with poor sputum conversion rates have been seen as well[17,18]. Incorporating the knowledge of minimum inhibitory concentration (MIC) with drug response over time [area under concentration curve (AUC 0-24)] and peak plasma concentration (C-max) of antituberculosis drugs may be better strategy to fight against delayed sputum conversion, drug resistance and relapses[24,25]. In individualized management strategies for a target population; TDM can be offered to improve the clinical outcome. But the cost of quantitative assay by high performance liquid chromatography (HPLC) and mass spectrometry are major deterrents. Cost outweighs the health gains by a TB patient especially in financially constrained countries. Logistic obstacles for TDM are frequent blood sampling, maintenance of cold chain and transport of blood samples to referral laboratories. Moreover, each analyte has specific reagent and quality control. Furthermore, decentralized setups, lack of specialized hospitals for tuberculous patients and pharmacokinetic laboratories with few qualified clinical pharmacologists for interpretation of these results[26,27] are all deterring standardization and optimum TB outcomes in our local population.

CONCLUSION

An early TDM monitoring has revealed low plasma INH concentration. Correction of dose to achieve expected plasma INH level will have promising time for sputum culture conversion. It will minimize the total statewide burden of slow responders and tuberculosis resistant cases.

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