ORIGINAL ARTICLE

Therapeutic Drug Monitoring of Isoniazid with Serum Hemoglobin LevelsiIn Pulmonary Tuberculosis Patients

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ABSTRACT

Background: Pulmonary tuberculosis is the most common infection among mycobacterial diseases in humans. Low plasma levels of first line anti-tuberculous drugs are related to drug resistance and therapeutic failure.
Aim: To determine the serum levels of isoniazid and hemoglobin in newly diagnosed pulmonary TB patients after 8 weeks of treatment.
Methodology: Newly diagnosed (30) pulmonary tuberculosis patients were enrolled to conduct the present study at Gulab Devi Hospital, Lahore-Pakistan. Blood samples were drawn at 02hrs and 06hrs post dose intervals for anti-tuberculous drugs. Written and informed consent was taken from patients. High performance liquid chromatography method using UV detection was carried out for determining plasma isoniazid levels after 8 weeks of anti-tuberculous drugs treatment.
Results: Plasma levels of isoniazid at C_2h were <1.5 µg/ml in 97.4% patients whereas 100% subjects had plasma levels of isoniazid at C_6h <1.5 µg/ml among Pakistani population during our research stud
Conclusion: We concluded that the therapeutic drug monitoring of isoniazid is imperative to rule out the cause of MDR-TB due to its low serum levels in Pakistani patients.
Keywords: High performance liquid chromatography, Isoniazid, Therapeutic drug monitoring,

INTRODUCTION

Among mycobacterial diseases, pulmonary tuberculosis is the leading infection in humans for hundreds of years. *Mycobacterium tuberculosis* (MTB) is the causative agent for this disease. It requires 6-24 months of treatment for its cure. The infection can affect other regions like lymphatics, pleura, bones, joints, meninges, spinal cord. This is known as extra-pulmonary tuberculosis[1]. Drug resistant cases are categorized as multidrug-resistant tuberculosis (MDR-TB) or extensively drug-resistant TB (XDR-TB). Pakistan is ranked 6th among countries for crowning number of multidrug-resistant (MDR) TB cases[2].

As shown by Global Tuberculosis Report 2015 that in 2014, 9.6 million people approximately had TB and 1.5 million died from it. Worldwide, it was estimated that there were new (3.3%) and previously treated TB patients (20.0%) were multidrug-resistant tuberculosis (MDR-TB) cases. This means that approximately 500,000 people developed MDR-TB in 2014. On average, roughly 9.7% patients with MDR-TB turned into extensively drug resistant TB (XDR-TB)[2]. Latent TB is present in about one-third of the world's population.

It's a droplet infection. Disease progression and transmission are influenced by many factors like number of bacilli in the droplets, the virulence of the bacilli, exposure of the bacilli to UV light, degree of ventilation and aerosolization[1]. It is more common in people with HIV, other co-morbidities and low socio-economical status.
Isoniazid (INH), one of the first line anti-tuberculous drugs for treatment of MTB used in fixed dose combination (FDC) with other drugs[3]. Treatment for MDR-TB needs those drug regimens that are prolonged (18-24 months), more efficacious and less toxic. Globally, treatment cure is just 50% as there are chances of relapse after completing the treatment for months or years[4].

In Pakistan, multiple reasons like poverty, less awareness about disease and its consequences, false beliefs about drugs being prescribed in TB clinics resulted in treatment failure. Instead of being eradicated, drug resistant strains of MTB have evolved that lead to MDR-TB with poor clinical outcomes[4]. Therapeutic drug monitoring (TDM) should be reviewed in current evaluation of severely ill TB patients. Low plasma levels of anti-tuberculous drugs are linked with drug resistance and therapeutic failure. Thus we have planned TDM of anti-tuberculous drugs to determine the serum levels of isoniazid and hemoglobin in newly diagnosed pulmonary TB patients after 8 weeks of treatment in current study.

MATERIALS AND METHODS

A descriptive study was held at University of Health Sciences (UHS) and Department of Chest Diseases, Gulab Devi Hospital, Lahore, Pakistan from January-December 2017 following their ethical committee approval. Newly diagnosed sputum positive pulmonary tuberculosis patients were admitted to the hospital and enrolled in the study after providing written informed consent. Fixed dose combination (FDC) protocol followed in Gulab Devi Hospital, Lahore was adopted. In accordance with the guidelines of the Pakistan National Tuberculosis Program, FDC form containing isoniazid (dose 300 mg) with other first line anti-tuberculous drugs were given daily to patients by strict monitoring for 8 weeks. Standardized meals were served to the subjects during study period. Pulmonary TB patients with both genders, age (18-65years) and MTB sensitive to 1st line anti-mycobacterial drugs were included in current

study. Pregnant females and patients with other co-morbidities and below 18 years of age were excluded from present study. Blood samples were taken at 02 and 06 hrs after drug administration on day1, 14 & 56. Separated plasma was first centrifuged then vortexed for 1 minute later stored immediately at −80°C till further use.

Statistical Analysis: Collected data was analyzed by Statistical Package for Social Sciences (SPSS software, version 20). Mean ± SD was given for quantitative laboratory parameters. Percentage was given for demographic parameters. Repeated measures ANOVA test was used to determine the mean differences in laboratory parameter and serum concentration among day 1, day 14 and day 56. Independent sample t test was used to determine the mean difference in serum concentration between 2 and 6 hour. A *p*-value < 0.05 was statistically significant.

RESULTS

Demographic parameters of 30 newly diagnosed pulmonary TB patients (Table 1).

On day1, at 2hr (C_2h) and 6hr (C_6h) serum INH levels (mean± SD) were 1.49 µg/ml ± 0.865 and 0.73 µg/ml ± 0.333 respectively. On day14, at 2hr (C_2h) and 6hr (C_6h) serum INH levels (mean± SD) were 1.39 µg/ml ± 0.918 and 0.56 µg/ml ± 0.485 respectively. Similarly, on day56, at 2hr (C_2h) and 6hr (C_6h) serum INH levels (mean± SD) were 1 µg/ml ± 0.474 and 0.39 µg/ml ± 0.409 respectively. Plasma drug levels decreased during treatment course. Results of mean serum INH(Fig.1)

Overall comparison among serum INH levels (mean ± SD) between C_2h (1.296 µg/ml ± 0.80) and C_6h (0.056 µg/ml ± 0.43) post dose in patients taking anti-tuberculous therapy reflected significant *p*-value <0.00019 (table: 2). This showed that serum drug levels change significantly between C_2h & C_6h intervals during whole study duration (Table 2)

Intraday judgment showed that C_2h and C_6h serum drug levels change with a pattern having a peak at 2hrs (C_2h) and trough at 6hrs (C_6h) on same day respectively with significant *p*-values <0.001* (Table 3).

Blood hemoglobin (Hb) was measured in the enrolled subjects three times i.e day1, 14 and 56. There was a mild change in Hb levels of subjects throughout study with insignificant *p*-value >0.259 (Table 4).

Table 1: Demographic parameters of patients taking anti-tuberculous therapy (n=30)

Parameters	Present	Absent
Family history	24%	76%
Immunization history	32%	68%
Sputum AFB (positive)	100	0
Gender percentage	Male 68%	Female 32%

Figure 1: Plasma INH levels on Day 1, 14 & 56 at 2 & 6 hrs post dose in patients taking anti-tuberculous drugs (n=30)

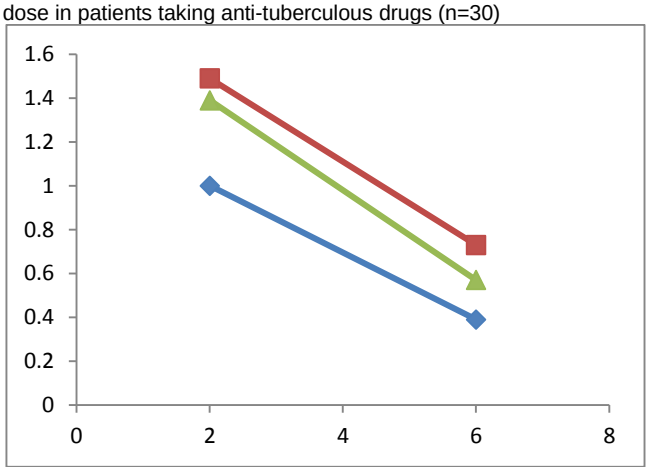

Table 2: Comparison of total serum INH levels at C_2h & C_6h in patients taking anti- tuberculous drug therapy

Hours	Mean (µg/ml)	Std. Deviation	Std. Error Mean
2	1.296	0.796	0.0919
6	0.056	0.432	0.0499

P value <0.0001* *Independent Samples Test = 7.008

Table 3: Intraday assessments of serum INH levels at C_2h & C_6h on day 1, 14 & 56

			Mean	Std. Deviation	Std. Error Mean	*p*-value
Day1	Concentration	2 hour	1.489	0.866	0.173	< 0.001*
		6 hour	0.733	0.334	0.067	
Day14	Concentration	2 hour	1.394	0.919	0.184	< 0.001*
		6 hour	0.565	0.485	0.097	
Day56	Concentration	2 hour	1.004	0.474	0.095	< 0.001*
		6 hour	0.390	0.410	0.082	

*All significant

Table 4: Blood hemoglobin levels of patients on anti-tuberculous therapy (n=30)

Days	Mean(mg/dl)	Std. Deviation
1	10.920	2.1434
14	10.716	1.9045
56	10.952	1.8844

P value 0.259
Insignificant

Where: Data presented in patients having % mean of hemoglobin ± SD (n=30)

DISCUSSION

Therapeutic drug monitoring of INH was planned to distinguish between MDR-TB cases or poor compliance. Therapeutic drug monitoring (TDM) for anti-tuberculous drugs is not usually performed in our setups. In the light of increasing multidrug resistant and extensively drug resistant tuberculosis cases, the use of TDM should be included in current treatment protocol of seriously ill TB patients. These anti-tuberculous health facilities work in colaboration with WHO. Treatment involves anti-tuberculous drugs in FDC as well as injectable form.

Our sample size was 30 as in other studies 12- 25 sample size is practiced[5]. In contrast, one study carried on Tanzanian population included 100 TB patients[6]. Both males and females were recruited in our work as in other studies. Females were 32 % while males were 68% as males are more affected by TB in Pakistan as well as globally. Selection of gender among subjects was paradoxical i.e 65% females and 35% males in one Iranian population[7,8].

In the current study, plasma samples of the patients were drawn on day1, 14 and 56 for C_2h and C_6h levels post-dose of anti-tuberculous drugs. Paradoxically, in one study held at Tanzania, samples were taken on two occasions at day 7 and 60 post–initiation at C_2h, C_4h and C_6h post-dose[6].

Concentrations of INH at C_2h were obtained to get peak serum concentrations, and concentrations at C_6h were done to check the rate and completeness of drug absorption. The target range of peak plasma concentrations of INH at C_2h has been reported was 3–6µg/ml[9]. INH concentrations between 2 and 3 µg/ml were considered low, and concentrations below 2 µg/ml were considered very low/ subtherapeutic[10]. In present study drug levels were categorized in the same lines as in above mentioned studies.

Most patients had drug concentrations below reference values, despite daily administration of anti-tuberculosis drug dosages. Plasma levels of INH at C_2h were <1.5 µg/ml in 97.4% patients whereas 100% subjects had plasma levels of INH at C_6h <1.5 µg/ml among Pakistani population during our reseach study. This variation in results may be due to genetic and ethnic variability among different populations. The prevalence of low plasma concentrations at various time intervals for anti-tuberculous drugs occurred commonly as estimated in previous studies[11]. In 2005, Tappero et al., conducted a study where 30% of patients showed low serum concentrations of INH whereas 26% had low concentrations of both INH and RMP[12].

In current study, we recorded serum hemoglobin levels of 30 enrolled patients on day 1,14 and 56 respectively. As Patients with pulmonary TB presented to us usually with anemia. Results showed that this parameter (Hb) changed insignificantly throughout the project. Similarly, same parameter was noted in other Indonasian study with similar results[13]. The compliance of patients for treatment was ensured by the hospital staff during stay in hospital.

CONCLUSION

All patients (30) had sub-therapeutic drug, isoniazid, plasma levels measured on day1, 14 and 56 at C_2h and C_6h leading to more burden of MDR-TB cases in Pakistani population.

Running head: Plasma isoniazid and hemoglobin level determination in TB patients.

Conflict of interest: None.

Funding: None

Limitations: We admit that our study had a number of limitations. It included too small sample size, inadequate blood samples on the same day, financial constrains with lack of resources. This study helped us to evaluate drug plasma levels as may be a leading cause to MDR-TB cases in Pakistani population.

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