

Isoniazid acetylation phenotypes in the Sudanese population; findings and implications

Monadil H. Ali^{a,b}, Alian A. Alrasheedy^{c,*}, Dan Kibuule^d, Mohamed Azmi Hassali^a, Brian Godman^{a,e,f}, Mohammed F. Abdelwahab^g, Raef Y. Abbadi^b

^a Discipline of Social and Administrative Pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM), Penang, Malaysia

^b Faculty of Pharmacy, Northern Border University, Saudi Arabia

^c Unaizah College of Pharmacy, Qassim University, Qassim, Saudi Arabia

^d School of Pharmacy, Faculty of Health Sciences, University of Namibia, Windhoek, Namibia

^e Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska Institute, Karolinska University Hospital Huddinge, Stockholm, Sweden

^f Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK

^g Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Egypt

ARTICLE INFO

Keywords:

Acetylation

Fast acetylators

INH

Isoniazid

Slow acetylators

Tuberculosis

Sudanese populations

ABSTRACT

Background: Isoniazid (INH) is the mainstay antimicrobial in the treatment of tuberculosis (TB). It is acetylated in the liver to acetyl-INH. However, there is variation in rate of acetylation of INH among TB patients (i.e. fast, intermediate or slow acetylators) which impacts on the treatment outcomes.

Aim: The isoniazid acetylation phenotypes in the expatriate Sudanese population were determined to provide future guidance since TB is prevalent in Sudan.

Methods: A community-based trial among Sudanese expatriates in Saudi Arabia was undertaken to identify INH-acetylation phenotypes. After overnight fasting, a single dose of 200 mg of INH was given to the volunteers. Three hours later, 5 ml of blood were drawn from each volunteer and prepared for High-Performance Liquid Chromatography (HPLC) analysis. The main outcomes were INH and Acetyl-INH concentrations in plasma and the subsequent Acetyl-INH/INH metabolic ratio (MR).

Results: The findings suggest that slow acetylation is highly prevalent among the study participants ($n = 43$; 84.31%). Moreover, there was no statistically significant correlation between age and the MR ($r = -0.18$, $P = 0.20$). Further, there was no significant association between gender and the MR ($P = 0.124$). Similarly, no significant association was found between smoking habits and MR ($P = 0.24$).

Conclusion: Isoniazid phenotyping suggests predominantly slow acetylation among the Sudanese in this sample. The study found no statistically significant associations between the MR and age or gender or smoking.

1. Introduction

Appropriate diagnosis and management of tuberculosis (TB) is a public health priority across countries [1,2], with TB still the second leading cause of death from infectious diseases world-wide including patients with concomitant HIV and TB [3–5]. The prevalence of TB in Sudan is a concern [6], with growing numbers of patients during the past five decades [7], although incidence rates are now decreasing [8]. This though may reflect under-reporting in conflict zones [9]. Overall, Sudan is considered a country with a high prevalence of TB, with 6587 cases reported in 2012 [7].

TB is also a concern in Saudi Arabia, although incidence rates have

decreased in recent years through a number of government initiatives, resulting in overall moderate prevalence rates [10–12]. Prevalence rates for TB do vary throughout Saudi Arabia and are generally higher among non-Saudis especially those coming from high prevalence countries which includes Sudan [10]. There is a relatively higher rate of multi-drug-resistant tuberculosis (MDR-TB) in Saudi Arabia compared with Western countries [11], which must be carefully managed with isoniazid a critical medicine although different strategies have been developed to manage MDR-TB [13–15].

The WHO recommends the 2HRZE/4RH regimen for new cases (pulmonary and extrapulmonary TB), which includes two months intensive treatment with four oral medicines, isoniazid (H), rifampicin

* Corresponding author.

E-mail addresses: alian-a@hotmail.com, aarshiedy@qu.edu.sa (A.A. Alrasheedy), dkibuule@unam.na (D. Kibuule), Brian.Godman@ki.se, Brian.Godman@strath.ac.uk (B. Godman).

<https://doi.org/10.1016/j.jctube.2019.100120>

(R), pyrazinamide (Z) and ethambutol (E), plus 4 months continuation phase with rifampicin and isoniazid. Isoniazid (H) and rifampicin are highly potent medicines to treat TB and they remain the cornerstone in the management of TB world-wide [16]. They are predominantly available together with other anti-TB medicines in fixed-dose combinations (FDC) [17,18]. Isoniazid can also be given as monotherapy for prophylaxis purposes [19], and is a low-molecular-weight medicine with a hydrophilic feature that can rapidly penetrate the gastrointestinal (GI) tract to reach the bloodstream [20].

The effectiveness of anti-TB medicines are predominantly influenced by two pharmacokinetic parameters, namely the area under the curve (AUC) and the maximum concentration (C_{max}) in the blood. Increased AUC value is proportionally correlated with the efficacy of TB drugs whereas low C_{max} can precipitate antimicrobial resistance [21]. The pharmacokinetic characteristics of isoniazid are further influenced by different patient-related factors including age, genetic status, comorbidities, and concomitant administered other medicines or food [22–24]. Isoniazid is converted to acetyl-isoniazid (Ac-INH) in the liver. In turn, Ac-INH is cleaved into iso-nicotinic acid and mono-acetyl hydrazine (MAH). The latter is further acetalized to form di-phenyl hydrazine [25]. Genetic polymorphisms currently show different frequencies with various ethnic backgrounds [20,26]. Many studies have indicated that the rate with which isoniazid is metabolized is controlled by two alleles as a single autosomal gene locus. Homozygous for a recessive allele determines slow acetylators whereas the homozygous or heterozygous dominant gene controls fast acetylators [27]. The routine incorporation of isoniazid into TB management protocols [14,18], and the influence of genetic variability in its metabolism, led to considering acetylation polymorphism [26]. Isoniazid acetylation status subsequently categorizes populations into slow, intermediate, or rapid acetylators [28], with the distribution of acetylation status (slow versus rapid acetylators) in the population being widely influenced by ethnic background as well as environmental factors [20,26].

Isoniazid undergoes hepatic acetylation via hepatic N-acetyltransferase 2 (NAT2) to be converted to a toxic substance which adversely impacts on the liver. Diversity in NAT2 plays a crucial part in liver impairment [29], and consequently must be carefully managed. Fast acetylators affect the bioavailability of isoniazid and subsequently confer drug resistance. Slow acetylators accumulate it beyond the therapeutic level which in turn harms some organs including the liver. Slow acetylation in particular has been found to be significantly associated with the risk of developing liver injury [30]. In fact, the incidence of INH induced hepatotoxicity is higher in slow acetylators [31,32]. For example, a study showed that the risk of developing INH hepatotoxicity was 22% for slow acetylators compared to 2.9% in fast acetylators [33]. This could be attributed to the higher accumulation of INH and acetylhydrazide (Ac-Hz) metabolite in slow acetylators [31,34]. In addition, fast acetylators are at a risk of liver toxicity [35]. This is a concern as most of the first-line anti-TB medicines including pyrazinamide and rifampicin also have harmful effects on the liver.

However to date, there appear to be few published articles concerning acetylation phenotyping among the Arab population [36]. We are aware that the rate of slow acetylators has been shown to be 72.3% among Saudi population using caffeine as a test substance [37] and the frequency of slow acetylators among Jordanians has been found to be 67.5% using dapsone as a test substance [38]. In contrast, a study involving 50 Egyptians using isoniazid as a test drug found the rate of acetylation at 82% [39], with a study conducted among 40 healthy Libyan male volunteers using sulphadimidine as a test substance showed the rate of slow acetylators was 65% [40]. In addition, among Moroccan TB patients where INH was employed as a test substance, the rate of slow acetylators was 61.8% [41].

Currently, it appears there is no information concerning acetylation phenotyping status using isoniazid as a test substance among the Sudanese population in the published literature. This is important given the high prevalence of TB in Sudan especially when combined with HIV

[7,8], the moderate prevalence of TB in Saudi Arabia which, as mentioned, is higher among some immigrant groups including those from Sudan [10], the relatively high rate of MDR-TB in Saudi Arabia [11], and the fact that the Sudanese population currently makes up 4% of the expatriate population in Saudi Arabia [42]. In addition, Anggraini et al. in Indonesia investigated the influence of acetylation status on the treatment outcome and serum concentration of isoniazid. Despite the lack of statistical significance perhaps due to low patient numbers ($n = 31$), the risk of conversion failure was approximately two fold greater among fast acetylators versus slow acetylators [43]. In view of this, it appears worthwhile to consider acetylation phenotyping of patients for individualization of isoniazid dosage regimens among the Sudanese population in Saudi Arabia.

Consequently, the purpose of the study was to describe the acetylation status among the Sudanese in Saudi Arabia using isoniazid as a test substance. In addition, the study aimed to examine whether there is association between acetylation status and age, gender and the smoking habits of participants. Understanding the acetylation phenotype of isoniazid and possible factors serves as a robust base for further studies to investigate factors correlated with the bioavailability that leads to resistance and precipitating the harmful effects of INH in a population with a high prevalence rate of TB. These findings can serve as a basis for further studies in Saudi Arabia as well as Sudan.

2. Method

2.1. Study setting

The study setting was community households located in Rafha in the northern borders of Saudi Arabia. Rafha is the second most populated city in the region where the population is entirely urban. The target population was healthy Sudanese residents who were living in Rafha during the study period. The total number of Sudanese residents in Rafha is estimated to be approximately 250 residents and whilst the entire population (i.e. 250) was targeted, fifty-one residents from both sexes subsequently volunteered to participate in the study. The study did not include TB patients as the TB patients in Rafha Central Hospital (RCH) were non-Sudanese during the study period.

2.2. Study design and data collection

A prospective open-label study was conducted among Sudanese volunteers residing in Rafha to principally measure phenotypes of isoniazid acetylation among this population. The selection criteria included volunteer blood donors with no history of any drug therapy within six months of commencement of the study. Patients with minor ailments but not on therapy and having no clinical or laboratory evidence of hepatobiliary, digestive, or renal disease were also included in the study.

2.2.1. Sample collection and preparation

The study took place between February to August 2017. After overnight fasting (from 12 midnight till 9 a.m.), each study subject received a single oral dose of 200 mg of isoniazid. Three hours later, a venous blood (5 ml) sample was drawn by a phlebotomist into an Ethylene Diamine Tetraacetic Acid (EDTA) tube and promptly centrifuged. The plasma was separated and frozen at -20 -degree Celsius for analysis. Collected samples were processed and analyzed in the research lab in the Faculty of Pharmacy, Northern Border University (NBU), Saudi Arabia. A single plasma sample taken 3 hrs post-INH oral dose of 200 mg is widely used in literature [36,44,45] to assess the extent of isoniazid acetylation. As a result of the current efficient essays methods a 200 mg is now considered suitable. Higher doses are no longer considered necessary, avoiding nausea and other adverse effects associated with INH [45].

2.2.2. Plasma concentration assays

The analysis for plasma concentrations for isoniazid and acetyl-INH was performed using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), Waters® 2545 Quaternary Gradient Module pump and equipped with Waters® 2998 diode array detector. This entire analysis system was controlled using Empower 3 Software. The column used in the system was water guard with asymmetry C18 of 5 μ m, 4.6 $\times$ 150 mm dimensions in an assembly of the Waters system (Waters Assoc., Milford, MA, USA). Five μ L volume of sample was injected using Hamilton syringe. The chromatograms for INH and acetyl-INH were obtained at 266 nm at room temperature with flow rate of 1.4 ml/min at 900 psi pressure. No interferences with the peaks from endogenous substances in the plasma were observed. Retention time (RT) was 3.8 min and 4.2 min for acetyl-INH and INH, respectively.

An isocratic mobile phase consisted of a mixture of 0.05 M ammonium acetate buffer (pH 6) and methanol (HPLC grade) (99:1, v/v), respectively with an elution rate of 1.2 ml/min. Two concentrations of ammonium acetate in water were administered as buffer solutions, 0.05 and 0.5 M adjusted by glacial acetic acid and sodium hydroxide to pH 6 and 8.2, respectively.

Acetyl-INH was synthesized in the lab by reacting isoniazid with acetic anhydride [46]. Various physical and chemical analytical methods including nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR), and the melting point characterized its structure and purity [36].

2.2.3. Calibration curves of INH and Ac-INH

Five calibration standards of isoniazid and acetyl isoniazid (Ac-INH) were prepared. Stock solutions of 1 g/L of pure INH and acetyl-INH were prepared using HPLC grade methanol. The working solution comprised 100 μ g/ml INH and 200 μ g/ml Ac-INH. Serial dilution with the geometry of 2 was adopted to yield 0.5-1-2-4 and 8 μ g/ml INH and 1-2-4-8 and 16 μ g/ml Ac-INH.

2.2.4. Preparation of plasma samples for HPLC

To 300 μ L of plasma, 250 μ L of 10% (w/v) trichloroacetic acid as a deproteinizing agent was added. The mixture was vigorously vortex-mixed at a rate of 10,000 rpm for 10 min. The trichloroacetic acid portion (supernatant) was taken and diluted at a ratio of 1:1 with ammonium acetate buffer (0.5 M, pH 8.20) to neutralize the excess acid [44].

Ultimately, the plasma samples were injected and analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) and the respective chromatogram peak areas of INH and Ac-INH were finally measured digitally by the detector in the apparatus. The relevant concentrations of both compounds were then calculated accordingly.

Plasma INH and acetyl-INH were determined by reverse-phase high-performance liquid chromatography (RP-HPLC). On injecting the working concentrations of both isoniazid and acetyl isoniazid (Acetyl-INH) into the HPLC, peak areas were obtained which are documented in Table 1. Pearson correlation coefficients were found significant ($P < 0.01$) with almost perfect correlation for both isoniazid and its metabolite, Acetyl-INH.

The acetylator phenotype was described by the histogram

Table 1

Peak areas values for the calibration curves of the working concentrations of isoniazid and Acetyl-INH.

INH conc. (μ g/ml)	Peak areas	Acetyl-INH conc. (μ g/ml)	Peak areas
0.50	6724	1	6655
1.00	14,884	2	13,414
2.00	32,300	4	31,112
4.00	70,532	8	55,214
8.00	168,903	16	91,248

NB: INH = isoniazid and Acetyl-INH = Acetyl isoniazid.

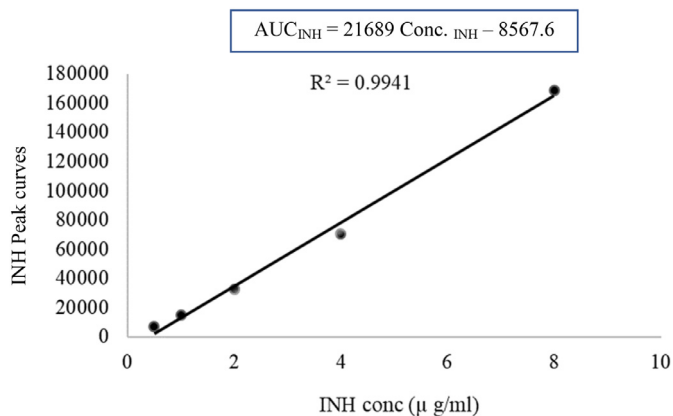


Fig. 1. Calibration curve of isoniazid solution.

distribution of the computed metabolic ratio (MR) of the levels of acetyl-INH to isoniazid in plasma. The subjects were subsequently classified based on the resultant histogram distribution. For every participant, the plasma concentrations of isoniazid and Acetyl-INH were calculated using the regression equations of both compounds. These equations were derived from the regression line of the calibration curves using the different concentrations prepared for this purpose.

The regression line equations that correlate the final concentration with the peak areas were computed for both isoniazid and Acetyl-INH. By least-squares method, a regression equation can be worked out (Figs. 1 and 2). The closer the correlation coefficient (Pearson) to unity, the more significant is the relation between the chromatogram peak area and the concentration. Consequently, the obtained linear equation can be used in any setting to determine unknown concentrations of INH and Ac-INH.

2.3. Statistical analysis

Data were entered and organized using MS-excel. Moreover, the data were then exported to IBM-SPSS version 24. The Pearson correlation test was used to examine the correlation between age and MR, whereas un-paired *t*-test was used to explore the association between gender, smoking status and MR.

2.4. Ethical approval

The study received ethical approval from the Committee of Bioethics at Northern Border University, Saudi Arabia with decision number (4/38/H) and dated 5/01/2017. The purpose of the study was clearly explained to the participants and written consents were then taken.

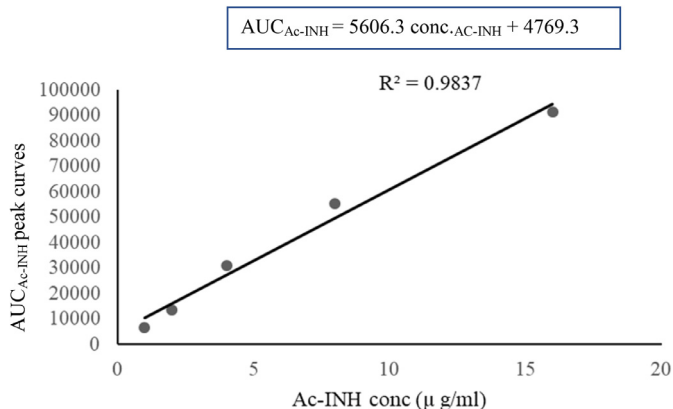


Fig. 2. Calibration curve of Acetyl INH solution.

Table 2

Plasma concentration of isoniazid & Acetyl-INH at 3 h post 200 mg single dose of Isoniazid.

Subject	Peak area (Ac-INH)	Ac-INH Conc. (µg/ml)	Peak area (INH)	INH Conc. (µg/ml)	Metabolic ratio – MR (Ac-INH Conc./ INH Conc.)
1	17,260	2.23	3991	0.58	3.84
2	17,425	2.26	6242	0.68	3.32
3	15,986	2.00	3976	0.58	3.44
4	29,622	4.43	5589	0.65	6.81
5	15,067	1.84	12,449	0.97	1.90
6	24,589	3.54	7261	0.73	4.85
7	24,189	3.46	7581	0.74	4.68
8	15,289	1.88	4583	0.61	3.08
9	20,850	2.87	1892	0.48	5.98
10	12,532	1.38	4733	0.61	2.26
11	10,849	1.08	2134	0.49	2.20
12	16,195	2.04	3943	0.58	3.52
13	16,917	2.17	3649	0.56	3.88
14	17,015	2.18	4830	0.62	3.52
15	9596	0.86	28,172	1.69	0.51
16	14,689	1.77	5252	0.64	2.77
17	15,334	1.88	4830	0.62	3.03
18	14,145	1.67	9105	0.81	2.06
19	13,919	1.63	7556	0.74	2.20
20	18,035	2.37	1245	0.45	5.27
21	19,494	2.63	8001	0.76	3.46
22	7459	0.48	9654	0.84	0.57
23	25,434	3.69	5690	0.66	5.59
24	22,628	3.19	7649	0.75	4.25
25	16,252	2.05	2817	0.52	3.94
26	9260	0.80	2959	0.53	1.51
27	29,158	4.35	1778	0.48	9.06
28	42,082	6.66	7650	0.75	8.88
29	15,125	1.85	232	0.41	4.51
30	13,472	1.55	4741	0.61	2.54
31	11,674	1.23	10,253	0.87	1.41
32	31,223	4.72	1432	0.46	10.26
33	51,916	8.41	2497	0.51	16.49
34	22,292	3.13	8800	0.80	3.91
35	12,659	1.46	13,032	1.00	1.46
36	39,755	6.24	2570	0.51	12.24
37	12,078	1.30	9736	0.84	1.55
38	12,323	1.35	15,288	1.10	1.23
39	24,234	3.47	15,829	1.12	3.10
40	16,844	2.15	15,419	1.11	1.94
41	11,620	1.22	11,841	0.94	1.30
42	10,890	1.09	8104	0.77	1.41
43	18,320	2.42	11,432	0.92	2.63
44	11,233	1.15	12,969	0.99	1.16
45	31,625	4.79	5672	0.66	7.26
46	20,763	2.85	18,626	1.25	2.28
47	23,166	3.28	13,732	1.03	3.18
48	25,662	3.73	11,602	0.93	4.01
49	38,060	5.94	6242	0.68	8.74
50	15,773	1.96	4724	0.61	3.21
51	22,348	3.14	17,471	1.2	2.62

NB: INH = isoniazid and Acetyl-INH = Acetyl isoniazid.

3. Results

3.1. Characteristics of study participants

Of the 51 healthy volunteers who participated in the study, 29 (56.9%) were male. The mean age $\pm$ SD of the participants was 34.3 ± 11.7 (range: 15–57) years. The mean age of males and females $\pm$ SD is comparable at 35 ± 11 years and 33 ± 13 years, respectively. The number of smokers to non-smokers in the study group was 43 (84.3%) and 8 (15.7%), respectively.

3.2. Main findings

The plasma concentrations of isoniazid and Acetyl-INH at 3 h post

Table 3

Metabolic ratios (MR) in different subpopulation of 51 volunteers following isoniazid intake.

	Mean (µg/ml)	S.E.M (µg/ml)	N
Overall MR	4.02	0.43	51
Gender			
Male	3.37	0.32	29
Female	4.87	0.89	22
Smoking habits			
Smokers	2.82	0.70	8
Non-smokers	4.24	0.49	43

SEM: Standard Error of the Mean.

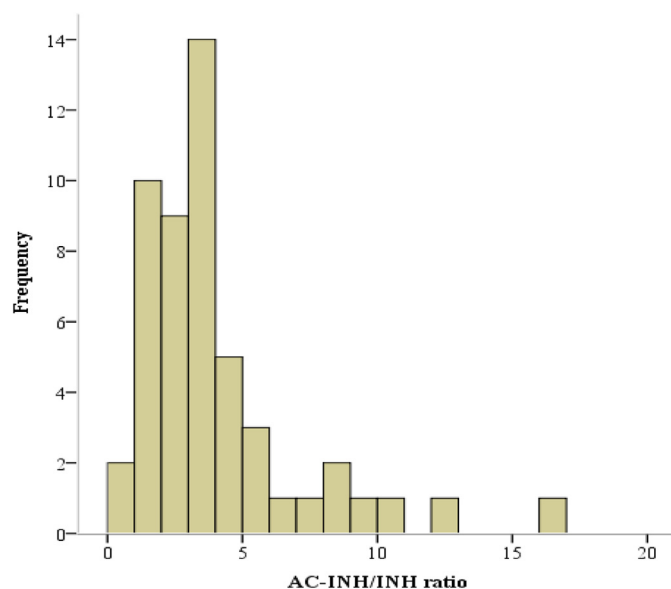


Fig. 3. Frequency distribution of plasma Acetyl-INH/INH ratios among ex-patriates Sudanese in Saudi Arabia.

200 mg of isoniazid are shown in Table 2.

The overall metabolic ratio (MR) $\pm$ SEM of the volunteers was 4.02 ± 0.43 (Table 3).

The unpaired *t*-test showed no significant association between gender and the metabolic ratio ($P = 0.124$) (Table 3). Similarly, no significant association was found between smoking habits (i.e. smokers versus non-smokers) and MR ($P = 0.24$).

The histogram (Fig. 3) showed bimodal distribution with an apparent antimode of MR of 6 separating slow and fast metabolizer groups. These findings show that slow acetylation is highly prevalent among the study participants ($n = 43$; 84.31%). Moreover, there was no statistically significant correlation between age and the Acetyl-INH/INH ratio ($r = -0.18$, $P = 0.20$) as shown in Fig. 4.

4. Discussion

We described the distribution of isoniazid acetylation phenotypes based on the distribution of metabolic ratio (MR), Acetyl-INH/isoniazid (Fig. 3). As mentioned, determination of acetylation by this method is considered a reliable and useful method and widely used in literature [36,41,44,45,47]. The results showed that the Sudanese volunteers in our sample were typically slow acetylators irrespective of their age. The study also showed that no significant association found between gender and MR, which is similar to the findings of Rasmussen and Brøsen in which gender did not impact on acetylation phenotypes status [48]. However, this is different to the findings of Asproдини et al. who found a significant association with gender [49]. We are not sure of the reasons behind the different results, and will be looking at this

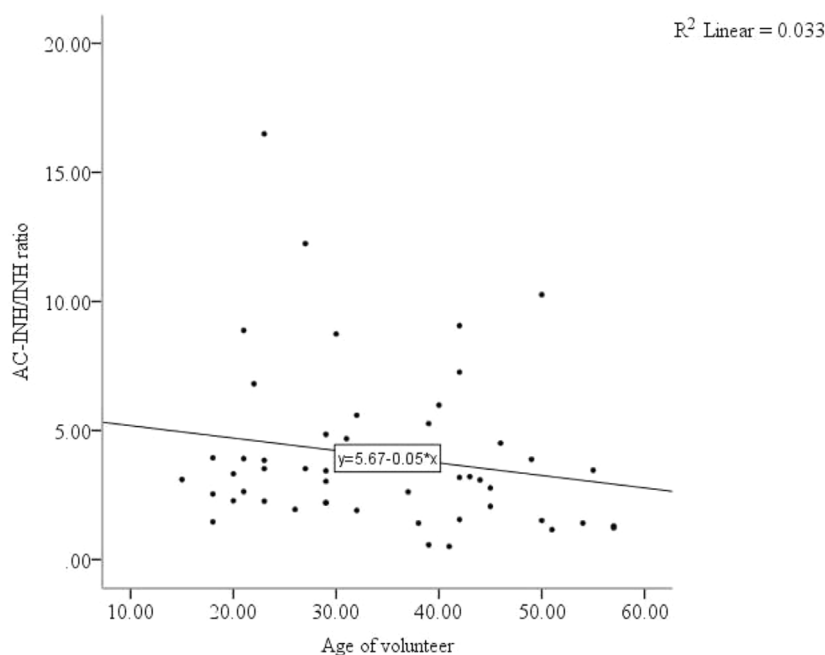


Fig. 4. Relationship between plasma Acetyl-INH/INH ratio and age of expatriates Sudanese in Saudi Arabia ($n = 51$, $r = -0.18$, $P = 0.20$).

further in future research.

It has also been shown that smoking is an enzyme inducer in different populations [50,51]. However, we did not find any correlation between smoking and MR, which is similar to the study of Asproдини et al. who also found no association between MR and smoking [49]. Again, we are not sure of the reasons behind these different findings, and will be investigating this further.

Similar studies carried out among Africans concluded that both slow and fast acetylators were prevalent among this population. Almost half of the recruited Senegalese patients (55.7%) were found to be slow acetylators in the study by Toure et al. [52], with comparable findings also reported by Touré A et al. in a study involving subjects from West Africa [53]. The rate of slow acetylators was reported to be 39% among children in South Africa [54]. These studies indicate the prevalence of slow acetylation among populations in Africa; however, the rates do vary depending on the population studied.

These findings contrast with other populations where the prevalence of slow acetylation can be low, e.g. as low as 14.6% and 13.1% among Indians and Japanese respectively and lower still (5%) among Alaskan Eskimos. In the French and German population, the prevalence of slow acetylation was reported to be 50% [44]. Out of 50 patients recruited in a Libyan study, 24 (48%) exhibited slow acetylation [44]. These findings supported the fact that the rate of acetylation varies from race to race; consequently, this needs to be taken into consideration in clinical practice.

Chamorro and colleagues investigated factors associated with the occurrence of anti-TB drug-induced hepatotoxicity (ATDH). Being slow acetylators negatively impacted on liver toxicity (OR, 2.615; 95%CI 1.264–5.411), another reason for determining the extent of slow acetylation among populations [55]. In addition, slow acetylators genotypes among Indonesian Malay ethnic group were found to increase the risk of cancer and liver injury [56], with, as mentioned, slow acetylation known to increase the risk of prostate cancer in Slovak populations [57].

In terms of practical implications, as the majority of participants are slow acetylators, the Sudanese may be at a higher risk of adverse effects associated with slow acetylation particularly liver injury. This indicates the need for more care in terms of patient counselling, close monitoring of adverse effects and follow-up when these patients are prescribed isoniazid. This is particularly important as most of the Sudanese TB

patients are working in remote areas in Saudi Arabia where contact with clinicians is relatively limited. TB drug resistance may also develop among Sudanese patients due to treatment default especially during the continuation phase of treatment. Consequently, individualization of therapy and regular follow up of TB patients are highly recommended in this population. In terms of therapeutic drug monitoring (TDM), whilst acetylation status for isoniazid is of no prognostic significance in daily dosing regimen, it may be of significance in twice weekly dosing [58,59]. However, the World Health Organization (WHO) current recommended regimen for TB is the daily regimen. In addition, given current limited resources in Sudan, we believe clinical monitoring of patients including incorporation of some investigations such as liver function tests (ALT and AST) is recommended as a more practical approach compared to TDM approach, which is hardly ever worthwhile for isoniazid [59]. These findings are also important as certain diseases including prostate cancer appear correlated with slow acetylators. The rate of prostate cancer was approximately three times higher compared with fast acetylators phenotype (OR, 2.91; 95% CI 1.43 – 5.94) in a Slovak population [57]. Consequently, it would appear worthwhile monitoring the risk factors associated with cancer.

Overall, we believe the study results could be of value for key stakeholders in both Sudan and Saudi Arabia as half a million Sudanese residents are living in Saudi Arabia [42]. Moreover, whilst the overall rate of MDR-TB among TB patients were 4.4% in a recent study by Al Ammari et al., 63.4% of the cases were reported among the non-Saudi population [11].

We are aware that there are a number of limitations with our study. These include the fact that there was only a small number of participants and these were volunteers. However, the sample size is comparable to other similar studies in literature. In addition, in terms of overnight fasting, the patients were instructed and well-informed not to eat the night prior to the testing. However, it cannot be confirmed that all patients strictly adhered to the overnight fast which could have resulted in delayed and decreased absorption, notably affecting a single time point plasma assessment. We also acknowledge that the volunteers did not have TB at the time. Consequently, a larger-scale study would be helpful to consider other pharmacokinetic parameters including C_{max} and t_{max} , as well as look more closely at the extent of slow acetylators among the Sudanese population including those with TB

and the implications. In addition, this study suggests that further exploration of NAT2 testing appears warranted in the Sudanese population

5. Conclusion

In summary, in this study, the vast majority of the Sudanese volunteers were slow acetylators. Moreover, acetylation of isoniazid was not associated with gender or smoking. Besides, no correlation was detected between age and MR. This needs to be taken in consideration in clinical practice when treating Sudanese patients with TB in Saudi Arabia, particularly clinical monitoring of adverse effects associated with isoniazid. In addition, this study suggests that further exploration of NAT2 testing appears warranted in the Sudanese population.

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

Acknowledgments

The authors acknowledge the staff of Northern Border University, Saudi Arabia who facilitated the processing of samples and instrumental analysis. Special thank you also goes to laboratory department and Pharmacy section, Rafha Central Hospital for the provision of isoniazid and heparinized tubes and other consumables.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical statement

This is to confirm that the study received ethical approval from the Committee of Bioethics at Northern Border University, Saudi Arabia with decision number (4/38/H) and dated 5/01/2017. The purpose of the study was clearly explained to the participants and written consents were then taken.

References

- [1] Sotgiu G, Sulis G, Matteelli A. Tuberculosis-a World Health Organization perspective. *Microbiol Spectr* 2017;5(1).
- [2] Elmadhoum WM, Noor SK, Bushara SO, Ahmed EO, Mustafa H, Sulaiman AA, et al. Epidemiology of tuberculosis and evaluation of treatment outcomes in the national tuberculosis control programme, river Nile state, Sudan, 2011-2013. *East Mediterr Health J* 2016 28;22(2):95-102.
- [3] WHO. Global Tuberculosis Report. 2016. Available at URL: <http://apps.who.int/iris/bitstream/10665/250441/1/9789241565394-eng.pdf?ua=1>. Accessed on 27th August 2019.
- [4] Oren E, Bell ML, Garcia F, Perez-Velez C, Gerald LB. Promoting adherence to treatment for latent TB infection through mobile phone text messaging: study protocol for a pilot randomized controlled trial. *Pilot Feasibility Stud* 2017;3:15.
- [5] Woimo TT, Yimer WK, Bati T, Gesesew HA. The prevalence and factors associated for anti-tuberculosis treatment non-adherence among pulmonary tuberculosis patients in public health care facilities in South Ethiopia: a cross-sectional study. *BMC Public Health* 2017 20;17(1):269.
- [6] Abdallah TM, Ali AAA. Epidemiology of tuberculosis in Eastern Sudan. *Asian Pac J Trop Biomed* 2012;2(12):999-1001.
- [7] Frahe EAM, Alkhalifa AA. Tuberculosis cases in Sudan; forecasting incidents 2014-2023 using box & jenkins arima model. *Am J Math Stat* 2016;6(3):108-14.
- [8] WHO. Tuberculosis report. Sudan 2017 Available at URL: https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2=SD&LAN=EN&outtype=html Accessed on 27th August 2019.
- [9] Hassanain SA, Edwards JK, Venables E, Ali E, Adam K, Hussien H, et al. Conflict and tuberculosis in Sudan: a 10-year review of the National Tuberculosis Programme, 2004-2014. *Confl Health* 2018;12:18.
- [10] Al-Hajj S, Varghese B. Tuberculosis in Saudi Arabia: the journey across time. *J Infect Dev Ctries* 2015;9(3):222-31.
- [11] Al Ammari M, Al Turaiki A, Al Essa M, Kashkary AM, Eltigani SA, Ahmed AE. Drug resistant tuberculosis in Saudi Arabia: an analysis of surveillance data 2014-2015. *Antimicrob Resist Infect Control* 2018;7:12.
- [12] Al Watban AZ, Al Salamah AA, El Faki MG. Prevalence of suspected tuberculosis in the Kingdom of Saudi Arabia according to conventional and molecular methods. *J Fam Community Med* 2014;21:1825.
- [13] Fregonese F, Ahuja SD, Akkerman OW, Arakaki-Sanchez D, Ayakaka I, Baghaei P, et al. Comparison of different treatments for isoniazid-resistant tuberculosis: an individual patient data meta-analysis. *Lancet Respiratory Med* 2018;6(April (4)):265-75. PubMed PMID: 29595509. Epub 2018/03/30. eng.
- [14] Rodriguez CA, Sasse S, Yuengling KA, Azzawi S, Becerra MC, Yuen CM. A systematic review of national policies for the management of persons exposed to tuberculosis. *Int J Tuberc Lung Dis* 2017;21(8):935-40.
- [15] Gegia M, Winters N, Benedetti A, van Soolingen D, Menzies D. Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. *Lancet Infect Dis* 2017;17(Feb(2)):223-34.
- [16] Short-Course Chemotherapy In Pulmonary Tuberculosis. A controlled trial by the British thoracic and tuberculosis association. *Lancet* 1975;305(7899):119-24.
- [17] Rée H. Treatment of tuberculosis: guidelines for national programmes (2nd edition). *Trans. R. Soc. Trop. Med. Hyg.* 1999;93(1):72.
- [18] Gallardo CR, Rigau Comas D, Valderrama Rodriguez A, Figuls M, Parker LA, Cayla J, et al. Fixed-dose combinations of drugs versus single-drug formulations for treating pulmonary tuberculosis. *Cochrane Database Syst Rev* 2016;17(May (5)):Cd009913.
- [19] Sia IG, Wieland ML. Current concepts in the management of tuberculosis. *Mayo Clin Proc* 2011;86(4):348-61.
- [20] Weber WW, David W. Clinical pharmacokinetics of isoniazid. *Clin Pharmacokinet* 1979;4(December(6)):401-22.
- [21] Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J Infect Dis* 2013;208(9):1464-73.
- [22] Ramachandran G, Hemanth Kumar AK, Bhavani PK, Poorana Gangadevi N, Sekar L, Vijayasekaran D, et al. Age, nutritional status and INH acetylator status affect pharmacokinetics of anti-tuberculosis drugs in children. *Int J Tuberc Lung Dis* 2013;17(6):800-6.
- [23] Mach J, Huizer - Pajkos A, Mitchell SJ, McKenzie C, Phillips L, Kane A, et al. The effect of ageing on isoniazid pharmacokinetics and hepatotoxicity in Fischer 344 rats. *Fundam Clin Pharmacol* 2016;30(1):23-34.
- [24] Seng K-Y, Hee K-H, Soon G-H, Chew N, Khoo SH, Lee LS-U. Population pharmacokinetic analysis of isoniazid, acetylisoniazid, and isonicotinic acid in healthy volunteers. *Antimicrob Agents Chemother* 2015;59(11):6791-9.
- [25] Meyer UA, Skoda RC, Grant D, Blum M. Genetic polymorphisms of drug metabolism. *Prog Liver Dis* 1990;9:307-23.
- [26] Evans DA, Manley KA, McKusick VA. Genetic control of isoniazid metabolism in man. *Br Med J* 1960;2(5197):485-91.
- [27] Lunde PKM, Frislid K, Hanslen V. Disease and acetylation polymorphism. *Clin Pharmacokinet* 1977;2(3):182-97.
- [28] Al-Yahyaee S, Gaffar U, Al-Ameri MM, Qureshi M, Zadjali F, Ali BH, Bayoumi R. N-Acetyltransferase polymorphism among Northern Sudanese. *BioOne* 2007;79(4):445-52.
- [29] Ben Mahmoud L, Ghazzi H, Kamoun A, Hakim A, Hachicha H, Hammami S, et al. Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatotoxicity in Tunisian patients with tuberculosis. *Pathol Biol* 2012;60(5):324-30.
- [30] Cho H-J, Koh W-J, Ryu Y-J, Ki C-S, Nam M-H, Kim J-W, et al. Genetic polymorphisms of NAT2 and CYP2E1 associated with antituberculosis drug-induced hepatotoxicity in Korean patients with pulmonary tuberculosis. *Tuberculosis* 2007;87(6):551-6.
- [31] Metushi I, Uetrecht J, Phillips E. Mechanism of isoniazid-induced hepatotoxicity: then and now. *Br J Clin Pharmacol* 2016;81(6):1030-6.
- [32] Walker K, Ginsberg G, Hattis D, Johns DO, Guyton KZ, Sonawane B. Genetic polymorphism in N-Acetyltransferase (NAT): population distribution of NAT1 and NAT2 activity. *J Toxicol Environ Health Part B* 2009;12(5-6):440-72.
- [33] Teixeira RLDF, Morato RG, Cabello PH, Muniz LMK, Moreira AdSR, Kritski AL, et al. Genetic polymorphisms of NAT2, CYP2E1 and GST enzymes and the occurrence of antituberculosis drug-induced hepatitis in Brazilian TB patients. *Memórias do Instituto Oswaldo Cruz* 2011;106(6):716-24.
- [34] Wang P, Pradhan K, Zhong X-b, Ma X. Isoniazid metabolism and hepatotoxicity. *Acta Pharmaceutica Sinica B* 2016;6(5):384-92.
- [35] Mitchell JR, Thorgeirsson UP, Black M, Timbrell JA, Snodgrass WR, Potter WZ, et al. Increased incidence of isoniazid hepatitis in rapid acetylators: possible relation to hydrazine metabolites. *Clin Pharmacol Therap* 1975;18(1):70-9.
- [36] Matar KM, Mayet AY, Ayoola EA, Bawazir SA, Al-Faleh FZ, Al-Wazzan A. Isoniazid acetylation phenotyping in Saudi Arabs. *J Clin Pharm Ther* 2004;29(5):443-7.
- [37] El-Yazigi A, Chaleby K, Martin CR. Acetylator phenotypes of Saudi Arabians by a simplified caffeine metabolites test. *J Clin Pharmacol* 1989;29(3):246-50.
- [38] Irshaid YM, al-Hadidi HF, Aburjeie MA, Rawashdeh NM. N-acetylation phenotyping using dapson in a Jordanian population. *Br J Clin Pharmacol* 1991;32(3):289-93.
- [39] Hashem N, Khalifa S, Nour A. The frequency of isoniazid acetylase enzyme deficiency among Egyptians. *Am J Phys Anthropol* 1969;31(1):97-101.
- [40] Karim AK, Elfellah MS, Evans DA. Human acetylator polymorphism: estimate of allele frequency in Libya and details of global distribution. *J Med Genet* 1981;18(5):325-30.
- [41] Ait-Moussa K, Hue B, Jana M, Begaud B, Soulaymani R. Determination of the acetylator phenotype in Moroccan tuberculosis patients using isoniazid as metabolic probe. *Int J Clin Pharmacol Ther* 2002;8:548-53.
- [42] Global Media Insight (GMI). <https://www.globalmediainsight.com/blog/saudi->

- arabia-population-statistics-2018/2018. Accessed on 27th August 2019.
- [43] Anggraini DI, Kristin Dwiprahasto I. The influence of acetylation status of tuberculosis patients on the isoniazid serum concentrations and sputum conversion after intensive phase therapy. *J Med Sci* 2018;50(1):59–69.
- [44] Tariq K, Almog IAM, Abdulfatah M, Gbaj, Omeran N, Fhid, Tuati Ali A. Isoniazid metabolism monitoring in Libyan patients using HPLC method. *J Chem Pharm Res* 2012;4(4):2204–8.
- [45] Hutchings A, Routledge P. A simple method for determining acetylator phenotype using isoniazid. *Br J Clin Pharmacol* 1986;22(3):343–5.
- [46] Fox HGJ. Synthetic tuberculosats VIII. Acyl derivative of isonicotinyl hydrazine. *J Organ Chem* 1953;18:1375–9.
- [47] Seifart HI, Parkin DP, Botha FJ, Donald PR, Van Der Walt BJ. Population screening for isoniazid acetylator phenotype. *Pharmacoepidemiol Drug Saf* 2001;10(2):127–34.
- [48] Rasmussen B, Brøsen K. Determination of urinary metabolites of caffeine for the assessment of cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activity in humans. *Ther Drug Monit* 1996;18(3):254–62.
- [49] Asproдини EK, Zifa E, Papageorgiou I, Benakis A. Determination of N-acetylation phenotyping in a greek population using caffeine as a metabolic probe. *Eur J Drug Metab Pharmacokinet* 1998;23(4):501–6.
- [50] Kalow W, Tang B-K. Caffeine as a metabolic probe: exploration of the enzyme-inducing effect of cigarette smoking. *Clin Pharmacol Therap* 1991;49(1):44–8.
- [51] Campbell ME, Spielberg SP, Kalow W. A urinary metabolite ratio that reflects systemic caffeine clearance. *Clin Pharmacol Therap* 1987;42(2):157–65.
- [52] Toure A, Cabral M, Niang A, Diop C, Garat A, Humbert L, et al. Prevention of isoniazid toxicity by NAT2 genotyping in Senegalese tuberculosis patients. *Toxicol Rep* 2016;3:826–31. 2016/01/01/.
- [53] Touré A, Diop C, Cabral M, et al. Study of NAT2 genetic polymorphism in West African subjects: example of an healthy non-smoker Senegalese population. *Mol Biol Rep* 2012;39(12):10489–96.
- [54] Schaaf HS, Parkin DP, Seifart HI, Werely CJ, Hesselings PB, van Helden PD, et al. Isoniazid pharmacokinetics in children treated for respiratory tuberculosis. *Arch Dis Child* 2005;90(6):614–8.
- [55] Chamorro JG, Castagnino JP, Musella RM, Noguera M, Aranda FM, Frías A, et al. Sex, ethnicity, and slow acetylator profile are the major causes of hepatotoxicity induced by antituberculosis drugs. *J Gastroenterol Hepatol* 2013;28(2):323–8.
- [56] Retno W, Susilowati KP, Intan R, Syukrini B, Rika Y. High frequency of NAT2 slow acetylator alleles in the Malay population of Indonesia: an awareness to the anti-tuberculosis drug induced liver injury and cancer. *Med J Indonesia* 2017;26(1):7–13.
- [57] Vilčková M, Jurečková J, Dobrota D, Habalová V, Klimčáková L, Waczulíková I, et al. Variation in N-acetyltransferase 2 (NAT2), smoking and risk of prostate cancer in the Slovak population. *Med Oncol* 2014;31(6):987.
- [58] Ellard G. Variations between individuals and populations in the acetylation of isoniazid and its significance for the treatment of pulmonary tuberculosis. *Clin Pharmacol Therap* 1976;19(5part2):610–25.
- [59] Ellard G. The potential clinical significance of the isoniazid acetylator phenotype in the treatment of pulmonary tuberculosis. *Tubercle* 1984;65(3):211–27.