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Isoniazid acetylation phenotyping in Saudi Arabs

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SUMMARY

Aims: **The present study is designed to investigate the acetylator status in Saudi Arabs.**
Methods: **Isoniazid (INH) acetylation phenotyping was studied in 136 Saudi Arabs in Riyadh, Saudi Arabia, using a single plasma sample taken 3 h post-INH oral dose of 200 mg. Metabolic ratio (MR) of plasma acetyl-INH (Ac-INH) to INH was used to determine the acetylation phenotype.**
Results: **The MR had a bimodal distribution with an antimode of 1·0. The frequency distribution of slow acetylators (MR < 1·0) was 94·9% ($n = 129$). Using Hardy–Weinberg Law, the gene frequency (q) of the recessive allele determining slow acetylator phenotype was found to be 0·97.**
Conclusion: **INH phenotyping suggests a high frequency of slow acetylators among Saudi Arabs. There was no association between the MR of plasma Ac-INH/INH and age or gender.**

***Keywords*: acetylator, phenotype, isoniazid, Saudi Arabs**

INTRODUCTION

Variation in the rate and pathways of drug metabolism by man is well known. Such variability may result from genetic or environmental influences. Genetic polymorphism of drug metabolizing enzymes may classify a population into subgroups which differ in their ability to biotransform certain drugs. The human acetylation polymorphism contributes to the variation in metabolism of certain drugs such as caffeine, hydralazine, procainamide, sulphonamides, clonazepam and isoniazid (INH) (1, 2).

With the introduction of INH therapy for tuberculosis, a genetic defect in its biotransformation led to the recognition of acetylation polymorphism (3). A bimodal distribution of acetylation of some drugs such as INH has been used to classify populations into rapid or slow acetylators.

Isoniazid is an anti-mycobacterial agent that still remains the mainstay of treatment of tuberculosis. Following oral administration, INH is rapidly and completely absorbed. It is metabolized mainly in the liver through acetylation to form acetyl-isoniazid (Ac-INH). The latter is then hydrolysed to iso-nicotinic acid and mono-acetylhydrazine (MAH). MAH is then acetylated to di-acetylhydrazine (4). The rate of acetylation of INH and MAH is genetically determined by acetylator phenotype as slow or fast distribution (3). Thus, individuals with deficient metabolism of certain drugs are referred to as slow metabolizers as compared with normal or extensive metabolizers (rapid metabolizers). Furthermore, it is now well established that genetic polymorphisms occur with variable frequency in populations with different ethnic backgrounds (3, 5). Several studies have indicated that the ability to metabolize INH is determined by two alleles as a single autosomal gene locus. Slow acetylation is homozygous for a recessive allele, whereas rapid acetylation is because of either homozygous or heterozygous dominant gene (6).

Several reports have described the frequency of acetylator phenotypes among populations of different ethnic and geographic location (7). For example, in Alaskan Eskimos, the frequency distribution of slow acetylators is 5% (1), whereas most of the Caucasian populations in Europe, particularly French and Germans, have equal

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proportions of slow and fast acetylators (8, 9). The Asians on the other hand, showed slow acetylator frequency distributions of 13·1% among Japanese (10), 14·6% among Indians (11) and 5% among Chinese (12).

The rate of acetylation is considered to be a critical determinant of therapeutic efficacy and toxicity of drugs which are metabolized by this pathway (3, 5, 6, 13). There is very little information concerning the acetylation pattern among Saudi population (14, 15).

The aim of this study was to assess the acetylator status among Saudi Arabs in Riyadh area (capital of Saudi Arabia) utilizing INH as a test drug.

METHODS

Of 136 subjects, 100 were volunteer blood donors who were asymptomatic (apparently healthy) and were on no drug therapy within 6 months prior to recruitment into the study. The remaining 36 were patients and were evaluated in King Khalid University Hospital (KKUH), Riyadh, Saudi Arabia, with minor ailments. They had no clinical or laboratory evidence of hepatobiliary, digestive or renal disease. They were not on any drug known to interfere with the disposition of INH. The age of the volunteers ranged from 18 to 75 years with a mean of 32·6 years. There were 67 males and 69 females. The study protocol was approved by the Ethics Committee of King Khalid University Hospital. The subjects were informed about the aim of the study and signed a written informed consent statement before entering the study.

After overnight fasting, each subject received 200 mg of INH as a single oral dose. After 3 h, venous blood (5 mL) was collected into heparinized tubes and immediately centrifuged. The plasma samples were then separated and kept frozen at −70 °C pending analysis.

Acetyl-INH was synthesized (16), and its structure and purity ascertained by various analytical methods including infra red (IR), nuclear magnetic resonance (NMR), and melting point determination. Plasma INH and Ac-INH were measured by a modified high-pressure liquid chromatography method (17). This involved a μ-bondapak C_{18} stainless steel column (10 μm, 300 × 3·9 mm I.D.) in an assembly of Waters system (Waters Assoc., Milford, MA, USA) consisting of a solvent delivery system (M-515), an autosampler (M-715) and a variable wavelength UV detector (M-486). The mobile phase was a mixture of methanol: 0·01 M phosphate buffer (5:95), adjusted to a pH of 6·9, and eluting at a rate of 2 mL/min. The eluent was monitored spectro-photometrically at 266 nm. Under these conditions, there were no interfering peaks from endogenous materials in plasma. The relative retention times of Ac-INH, INH and nicotinamide, the internal standard (IS) were 4·1, 5·2, and 6·9 min, respectively. Assay calibration was by analysis of INH and Ac-INH-spiked human plasma samples and plotting a graph of peak area ratio (INH or Ac-INH with respect to the IS) against the drug or its metabolite concentration. Intra-day coefficients of variation for INH and Ac-INH were 4·6 and 5·8%, respectively. The limit of detection of INH and Ac-INH was 50 ng/mL for both compounds.

Acetylator phenotype was determined from the metabolic ratio (MR) of Ac-INH to INH in the plasma sample. Subjects with MR < 1·0 were then classified as slow acetylators and conversely, MR > 1·0 as fast acetylators (18).

RESULTS

Frequency histograms of MR of Ac-INH to INH showed an apparent bimodal distribution with an apparent antimode of acetylation ratio of 1·0 separating slow from fast acetylators (Fig. 1).

Of 136 subjects studied, 129 (94·9%) were slow acetylators and six (5·1%) were fast acetylators. The latter were healthy volunteer blood donors. The gene frequency (q) of the recessive allele determining the slow acetylator phenotype was calculated as 0·97 using Hardy–Weinberg Law.

Moreover, as shown in Fig. 2, there is a lack of correlation between the plasma Ac-INH/INH MR and the age of the subjects (r = −0·0748).

DISCUSSION

During the past four decades, a considerable amount of information has accumulated on the distribution of acetylator phenotypes among populations of different ethnic and geographic origin. The proportions of rapid and slow acetylators vary considerably between different populations in relation to ethnicity and geographic location (7).

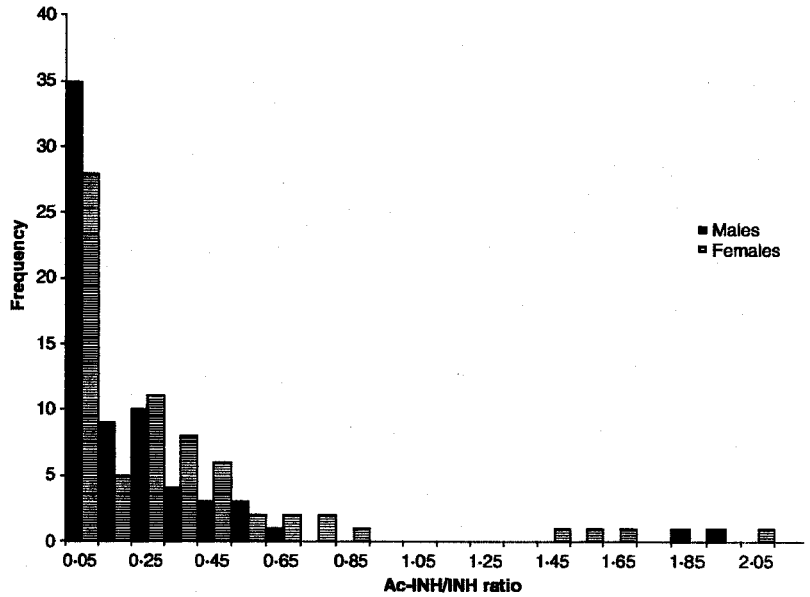

Fig. 1. Frequency distribution of plasma Ac-INH/INH ratios in Saudis.

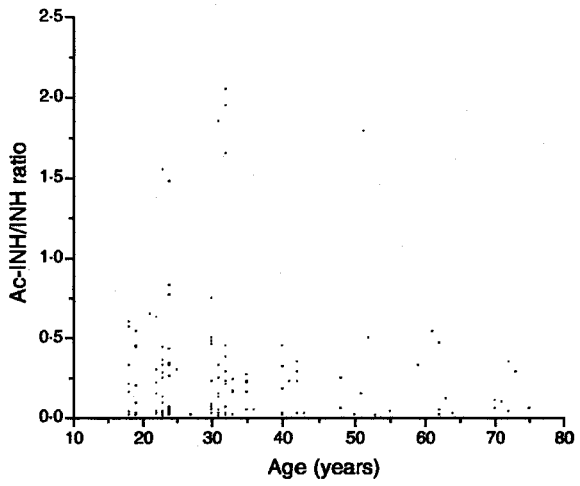

Fig. 2. Relationship between plasma Ac-INH/INH ratio and age of Saudi subjects (n = 136, r = −0·0748).

Despite the extensive reports covering various ethnic groups, reports on the pattern of acetylation in Arabs are very scanty. The frequency distribution of slow acetylators in a sample of Jordanians was found to be 67·5% using dapsone as a test drug (19). In contrast, a study involving 50 Egyptians, suggested a frequency of slow acetylators of 82%, using INH, with a slow acetylation gene frequency of 0·91 (20). For Libyan Arabs, slow acetylators were found to be 65% using sulphadimidine (21). For Moroccan Arabs, the frequency of slow acetylators was found to be 61·8% (22), and 90% in another study (1), both using INH as a test drug. On the other hand, the earlier studies involving Saudi Arabs showed frequency of slow acetylators of 63·4%, using sulphamethazine as a test drug (14), and 72·3% utilizing caffeine metabolite as a test drug (15).

Our study confirms the high frequency of slow acetylation in the Saudi Arab population (94·9%; gene frequency of 0·97), irrespective of their geographical locations. The explanation for the high gene frequency for slow INH acetylation among Saudi Arabs may not be restricted to their ethnic origin but also to the high rates of consanguineous marriage. The present study was conducted in the central area (capital city) of Saudi Arabia, with a fairly homogenous sample. Whether other tribal populations within the Kingdom would reflect a similar pattern needs further investigation. A previous study involving the acetylation status of sulphamethazine of both rural Bedouins and urban dwellers, reported a frequency of 63·4% (gene frequency of 0·8) of slow acetylators. This may reflect the heterogenous sample population (14).

The present findings contrast sharply with the observations of Iselius and Price-Evans who reported a significant effect of age and gender on INH plasma levels (23). However, our findings are in agreement with previous studies on gender effects (24).

The therapeutic and epidemiologic implications of INH acetylation phenotype are becoming more apparent. The acetylation polymorphism has

important consequences for both drug therapy and xenobiotic toxicity. It has been shown that in patients treated with INH and rifampicin, the incidence of jaundice and possibly raised serum alanine aminotransferase, are higher in subjects with the slow INH acetylator phenotype (25–27). Earlier studies have suggested more frequent occurrence of phenytoin toxicity in patients concurrently being treated with INH (28, 29). This combination is most commonly prescribed among tuberculosis patients in Saudi Arabia but the incidence of hepatotoxicity is not well defined.

Furthermore, associations between acetylator phenotype and diseases such as cancer of the bladder, Gilbert's disease, leprosy and early development of thyrotoxicosis have also been reported (4, 6, 7, 20). Slow acetylators are also reportedly at higher risk of INH adverse effects such as peripheral neuritis and hepatic toxicity (7, 30, 31). Phenotyping the acetylator status of patients for appropriate individualization of dosage regimen appears worthwhile. Bladder cancer is common in the Saudi population and a report from a tumour registry indicated that in the male, it ranked fifth in frequency when compared with other solid tumours after lung, oral cavity, CNS and liver tumours (32). It has been suggested that carcinogenic amines are substrates for N-acetyltransferases. Similarly, the age of onset of thyrotoxicosis among Saudi patients is relatively younger when compared with Caucasian patients (33). Although the exact mechanism and significance of these associations still require further studies, it is prudent to exercise caution in Saudi population treated with INH and other similar drugs in order to avoid unwanted drug interaction.

With regard to the influence of slow acetylation on INH efficacy, it may be predicted on the basis of our findings that in Saudi patients, who are almost universally slow acetylators, treatment failure due to inadequate blood levels should be rare. However, this needs confirmatory evidence from appropriate efficacy studies.

CONCLUSION

The vast majority of Saudi Arabs are slow acetylators. While the estimate of 94·9% is higher than values reported in other studies the consistent preponderance of slow acetylators among Arabs, irrespective of geographic location is confirmed.

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