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N-acetylation Phenotyping using Isoniazid in an Iranian and an Afghani Population

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The N-acetylation of isoniazid was studied in three different groups of Iranian and Afghani population (182 healthy volunteers, 78 infectious non-tuberculotic patients and 111 tuberculous patients, total of 371 subjects).The frequency of slow acetylator was determined using the percentage of acetylisoniazid excreted in urine which was about 59% in both populations. Also, the frequency distribution histogram of isoniazid phenotypes in studied population showed an apparent bimodal pattern. Statistical analysis of healthy volunteer's data versus all other patient's data indicated that there is no significant difference between slow and rapid acetylators.The finding of this study is very similar to that of the earlier results found among other cauccasian groups. However,The knowledge of the acetylator phenotype of a patient can help to determine the relative risk for some of the drug-related toxic and therapeutic responses.

Genetic differences in the ability of individuals to metabolize a drugthrough a given pathway are recognized as an important contributor to the large interindividual differences in biotransformation within a population[1]. One of the best known examples of genetic sources of variability is N-acetylation polymorphism[2]. Acetylation exhibits a genetically controlled distribution within any given population. Individuals can be phenotyped as either slow or rapid acetylator using a test drug e.g. clonazepam, dapson, isonizid, phenelazine and sulphadimidine[2-6]. The slow acetylator phenotype is inherited as an authosomal recessive trait and shows a distribution that is highly racedependent[7]. The frequency of slow acetylation phenotype is 13% in Chinese[8], 6.6% in Japanese[9], 57.4% in Spanish[10], 62.2% in USA[11], and 90% in Moroccan[2], populations. Owing to drug accumulation it is expected that slow acetylators would have higher incidence of toxic effects to drugs that are mainly metabolized by acetylation. On the contrary, fast acetylators may exhibit therapeutic failure after standard doses. These expectations are more likely to occur at the extremes of slow and fast acetylatior distribution. In addition, acetylator status may serve as a marker for certain diseases or their complications, Slow metabolisers are more prone to develop peripheral neuropathies when given isoniazid[12].

It is claimed that slow acetylators are at a greater risk of developing hydralazine and procainamide-induced systemic lupus erythematosus[2,10]. In addition, it has also been suggested that the slow acetylator phenotype may be associated with a greater incidence of bladder cancer, especially in individuals exposed to high levels of arylamines[11]. Thus it can be envisaged that genetic polymorphism of N-acetylation can have a direct bearing on the clinical toxicity of drugs. Therefore, identifications of the acetylator status of an individual is necessary in order to provide adequate drug therapy with minimal or no toxic effects, especially during chronic treatment with drugs known to undergo acetylation as major metabolic pathway.

Isonizid is still considered the most important drug worldwide for treatment of all types of tuberculosis and it is also used as a test drug for determination of the acetylator phenotype[13-14]. The frequency of slow acetylator status in Iranian and Afghani population has not been evaluated. Thus the main purpose of this study was to determine this frequency in three different groups of Iranian population and also in a minor Afghani population

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Table 1 - Comparison of slow and fast acetylators in three different groups of volunteers

Volunteers	Total no.	Slow acetylator	Fast acetylator
Healthy Subjects	182	113	69
Infectious Patients	78	46	32
Tuberculotic Patients	111	61	50
Total	371	220	151
(%)		(59.3%)	(40.7%)

(mainly from western part of Afghanistan), using isoniazid as a test drug.

MATERIAL AND METHODS

Subjects and Procedures:

A total of 371 subjects (129 females and 242 males, 9 to 82 years old), 182 healthy volunteers, 78 infectious (non-tuberculotic) patients and one hundred and eleven tuberculotic patients participated in the study. The subject were instructed of the objective of the study. All the subject were physicaly examined by physician and a blood sample were taken before the study to determine the levels of most seroimunologic factors. The patient was taken off all drugs for two days before the test. In order to confirm that the treatment had, in fact, been discontinued, a specimen of urine was collected just before the administration of isoniazid and examined for the presence of isoniazid and actylisoniazid. A test dose of 600 mg isoniazid, in the form of tablets, (2 x 300 mg tablets, isoniazid tablet batch No. 924 Hefa-Frenon, D 4712, ST IV A., Germany), was administered orally. At the sixth hour after ingestion, subject were asked to void urine and the urine passed between 6 and 8 h, was collected. The urine samples, were stored at-20° for a period not exceeding one week.

Assay procedure:

Isoniazid and acetylisoniazid in urine samples were estimated by a colorimetric method[15]. All samples were analyzed in duplicate by this colorimetric method. A SP6-200 spectrophotometer, Pye Unicam was employed for analysis of the samples. All the chemicals used were of analytical grade. Procedure is carried out on two aliquots (A and B) of each urine sample. In aliquot A, only the acetylisoniazid excreted in urine is determined, while in aliquot B total hydrazides i.e. the acetylisoniazid excreted and the isoniazid acetylated *in vitro* are estimated together.

For classification of patients into slow and fast inactivators, per cent acetylisoniazid excreted in urine is estimated. Those with a percentage of less than 70 are identified as slow inactivators;those with values of 70 or greater are fast inactivators[15].

Statistical analysis:

Differences between group means were assessed by the unpaired Students t-test and considered significant when the P value was ≤ 0.05.

RESULTS AND DISCUSSION

Since in some cases urine samples were stored in the deep freezer at-20°, sometimes for about one week before their isoniazid and acetylisoniazid concentrations were estimated, the stability of isoniazid in urine during storage was investigated. The isoniazid and acetylisoniazid concentration of six urine samples with different concentration of acetylisoniazid (10-60 mg/l) and 761 mg/1 isoniazid were assayed immediatley after preparation. All these samples were stored at-20° and again analyzed five month later. The results of these experiments was compared. Statistical analysis between two sets of results showed no significant difference. Therefore it can be pointed out that isoniazid and acetylisoniazid in this condition are stable for a period of at least five months.

As indicated earlier 371 subjects of three different groups, were studied. The results of this study are summarized in Table 1. The results of determinations of the rate of inactivation of isoniazid in Iranian population has been divided into two groups of rapid and slow inactivators. The frequency distribution histogram of the acetylisoniazid to isoniazid ratio is shown in Figure 1. The histogram shows an apparent bimodal distribution.

Table 2 - Comparison of slow and fast acetylators between Iranian and Afghani population

Subjects	Total no.	Slow acetylator	Fast acetylator
Iranian	317	189	128
(%)		(59.6%)	(40.4%)
Afghani	54	32	22
(%)		(59.2%)	(40.8%)

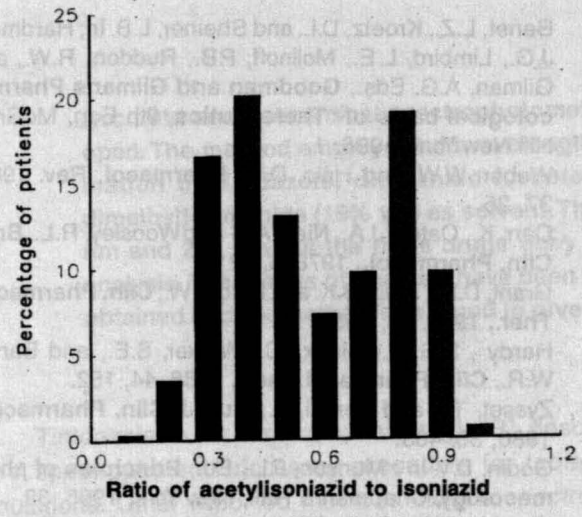

Fig. 1 : Distribution of isoniazid phenotypes in Iranian population

In this study, 59 per cent of subjects were classified as slow inactivators and 41 per cent of them were classified as rapid acetylators. The results of studies carried out among tuberculotic, infectious (non-tuberculotic) patients and healthy volunteers showed no significant differences between two different types of inactivators in three mentioned groups of subjects. The overall proportion of slow acetylators among the Iranian population (in this study 59%) is very similar to the proportions found among other Caucasian groups[16]. Statistical analysis of data from healthy volunteers versus data from all tuberculotic and infectious (non-tuberculotic) patients indicated that there was no significant difference between slow and rapid acetylators.

The relation between the level of seroimmunologic factors and inactivator phenotype of isoniazid were investigated. Results indicated no significant difference between the mean level of seroimmunologic factors (white blood cell, primed lymphocyte typing, prothrombin time, partial thromboplastin time, glucose, ceratinine, triglyceride lactate dehydrogenase,serum glutamic-oxalocetic transaminase, serum glutamate pyruvate transaminase) in two groups of slow and rapid acetylators. Although, there was significant differences between the levels of factors such as, erythrocyte sedimentation rate, blood urea nitrogen and cholesterol,it can be pointed out that there is no relationship between phenotype of isoniazid and seroimmunologic factors level.

Table 3 - Distribution pattern of slow acetylators in south Indian and Caucasian population in different countries[16]

Population	No. studied	Slow acetylators (%)
South Indian : Madras	1,477	59
Caucasian		
USA	200	52
USA	105	58
Germany	524	57
Finland	341	58
Britain	135	62
Sweden	130	68
Czechoslovakia	421	60
Canada	102	59
Afghanistan	54	59
Iran	317	59

There were nine hepatitis patients among the subjects, five of them rapid and four of them slow acetylators. Although the number of hepatitis patients was not enough, but statistical analysis showed no significant differences between percentage of phenotype in hepatitis and non hepatitis population.

Fifty four Afghani subjects were tested against 121 Iranian subjects. Results of this evaluation are very close as indicated in Table. 2. Statistical analysis showed no significant differences between rapid and slow acetylators in both these populations. It should be pointed out that these results are not unexpected because Iranian and Afghani (mainly state of Harat) people has the same racial origin.

The finding of this study confirmed earlier results of studies carried out among tuberculous patients or healthy individuals of South Indian and/or Caucasian origin[17]. The overall proportion of slow acetylators among the Iranian and Afghani population studied (about 59%) are very similar to the proportion found among the south Indian population and all other Caucasian groups studied (Table 3). There is no significant difference between slow and rapid acetylators in different Iranian population studied. There was also insignificant differences between the levels of nearly all seroimmunolgic factors and inactivator phenotype of isoniazid. The present findings are important and often useful for various clinical purposes.

The clinical consequences (therapeutic and toxic) of drug acetylation polymorphism showed that genetic show acetylators are more likely than rapid acetylators to experience adverse drug reactions[12,18]. The results of several different clinical trails have shown that the isoniazid acetylator status of tuberculotic patients treated with isoniazid containing regimens, is of no prognostic significant when treatment is given on a daily basis. It may, however, be of significance when once and/or twice-weekly regimens are employed, especially in circumstances in which only a short period of initial daily chemotherapy is given and where the companion drug employed during these phases is relatively weak[16].

However rapid acetylator may show a diminished therapeutic response if isoniazid is administered on a once-weekly basis. One-weekly isoniazid containing regimens must therefore be considered as unsuitable for general use, particularly for treating patients from races with predominantly rapid acetylators. Thus, knowledge of the acetylator phenotype of a patient can help to determine the relative risk for some of the drug-related toxic and therapeutic responses.

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