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ACETYLATOR PHENOTYPE DETERMINATION OF HEALTHY AND TUBERCULOSIS AFFECTED POPULATION OF PUNJAB

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Abstract: The acetylator phenotyping study was conducted on healthy human volunteers (n 120) and diagnosed pretreatment tubercular patients (n 60). Isoniazid was used as test drug in healthy individuals, while sulphamethazine served the same purpose in proposed tuberculosis patients. Urine and blood samples were assayed spectrophotometrically for drugs and their metabolites. The percentage abundance of fast acetylators in both healthy and tubercular patients was 43.33% and 41.67% respectively. Among the population of Punjab 42.7% were proved fast, while remaining 57.3% were slow acetylators.

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INTRODUCTION

The genetically determined polymorphic acetylation of drugs like, isoniazid (INH), procainamide, hydralazine, dapsone, sulphamethazine (SMZ), and certain other drugs containing hydrazine or aromatic amino groups has been studied by several investigators[1-4]: Out of these isoniazid is an important drug used in the treatment of tuberculosis[5].

The rate of N-acetylation of isoniazid (INH) and other drugs in controlled by N-acetyl - transferase, which is present in the liver and intestinal mucosa[6]. Within populations, the acetylation capacity displays a bimodal distribution, enabling the classification of subjects either fast or slow acetylators[7]. This has clinical implications as certain major adverse effects are more frequent among slow acetylators rather than fast acetylators. Slow acetylators have been reported to be at greater risk of developing drug induced systemic lupuserythematosus (S.L.E) and isoniazid induced polyneuritis, while fast acetylators produced higher levels of N-acetylated metabolites, some of them are biologically active or act as a reactive intermediates in pathways with toxic end products[8-10]. Slow acetylators are homozygous for a recessive gene while fast acetylators are either homozygous or heterozygous for a dominant gene. Thus three phenotypes can be separated as slow homozygotes, rapid homozygotes and intermediate heterozygotes[1,11,12] Great racial and geographical difference in ability to acetylate isoniazid, dapsone, and sulphamethazine have been found[3,4,13-15]. Japnese, Korean, Eskimos and Lapps have high proportion of rapid acetylators, while proportion is much lower in Causcasians. Indians are, however, 58% slow acetylators and 42% fast acetylators[16].Present study was undertaken to determine the acetylator status of Pakistani (Punjabi) population and make comparison with other nations.

MATERIALS AND METHODS

Chemicals

Isoniazid was donated by Willshire Laboratories, While acetylisoniazid (Ac-INH) was synthesized by method of Mitchell et al[17]. Sulphamethazine was purchased from Sigma Laboratories while acetylsulpphamethazine (Ac-SMZ) was synthesized by the method of Chapron et al [12]. All other chemicals used were of analytical grade purchased from Sigma, BDH and Merck Laboratories.

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Volunteers Selection

The volunteers were divided into two groups A & B. Group A contained 120 healtthy individuals belonging to different regions of Punjab (Pakistan) and with different ethnic groups. All the volunteers in this group were the students of Bahauddin Zakariya University, Multan, ranging 18 to 30 years in age and 50 to 80 Kgs in body weight (40 were females and 80 males). None of them have been suffering from any recogniseable disease and no body was taking any medication before our study. Group B contained 60 tuberculosis patients (16 females and 44 males) admitted to Chest Diseases Ward, Nishtar Medical College Hospital, belonging to different regions of Punjab (Pakistan). The blood samples were withdrawn from forearm vein when patients were freshly admitted to the hospital before starting the regular treatment of tuberculosis. They ranged 25 to 60 years in age and 50 to 70 kg in body weight.

Drug Administration

To group A,isoniazid (INH) was administered as an oral dose (Isonex Tablets) of 7.5 mg /kg body weight with a glass of water (300 ml) after overnight fasting. Nothing was given orally during 2 hours after drug administration. In Group B sulphamethazine was given as an oral dose (15 mg/kg body weight) after overnight fasting with a glass of water (300 ml).

Sampling Procedure

In group A heparinized blood samples (4ml) were withdrawn from forearm vein at 0, 1 and 3 hours after drug administration with the help of disposable syringe. The blood samples were centrifuged, plasma separated and stored (-20°C) in serum tubes. Urine samples were collected at 0, and 3 hours after drug adminisstration, volume was measured and an aliquot (20 ml) stored (-20°C) in plastic bottles.

In group B heparinized blood samples (4ml) were withdrawn from forearm vein at 0, and 6 hours with the help of disposable syringe, plasma was separated by centrifugation and stored (-20°C) in serum tubes.

Drug Analysis

Isoniazid and acetylisoniazid were analysed by colorimetric procedure [18] using Shimadzu-UV-Visible Spectrophotometer. Similarly Suphamethazine and acetylsulphamethazine were analysed by Bratton and Marshall method [19], colorimetrically.

Phenotyping Procedure

In group A acetylator phenotype was determined by the method of Hutchings and Routledge [20] using acetylisoniazid/isoniazid (Ac-INH/INH) ratio (at 3 hours) in plasma samples. However Ac-INH/INH ratio in urine (0 to 3 hours) was also used as determinant of acetylator phenotype for confirmation of results [21]. A new method was developed, which proved an easy method of acetylator phenotyping using acetylation percentage (0 to 3 hours urine) as determinant of acetylator status.

Acetylator phenotype of group B subjects was determined by using acetylation percentage of sulphamethazine (SMZ) in blood at 6 hours[22]. Acetylation percentage was calculated by the following formula:

$$\text{Acetylation Percentage} = \frac{\text{Acetyl-metabolite}}{\text{Total drug*}} \times 100$$

*Total drug = Free drug + Acetyl - metabolite.

RESULTS

The results of group A indicate that out of 120 volunteers 43.33 % are fast (n = 52) and 56.67 % slow (n =68) acetylators (Table 1, Fig.1). Table 1 lists the mean plasma concentrations of isoniazid (INH) and acetylisoniazid (Ac - INH) at 1 and 3 hours. This also illustrates the acetylisoniazid/isoniazid (Ac-INH/INH) ratio (at 3 hours in plasma). Frequency distribution histogram (Fig.1) indicates that value of acetylisoniazid/isoniazid (Ac-INH/INH) ratio exhibit bimodal distribution pattern among volunteers studied. This distribution discriminated fast and slow acetylators.

Table 1

Plasma Concentrations of Isoniazid, Acetylisoniazid and Percentage of Fast and Slow Acetylators in Population of Punjab

		Mean plasma concentrations (μg/ml)	
Acetylator Phenotype*		Fast Acetylators (n = 52)	Slow Acetylators (n = 68)
	Time (hrs)		
Isoniazid	1	8.47 ± 0.85 (μg/ml)	9.49 ± 0.83 (μg/ml)
	3	3.64 ± 0.06 (do)	5.66 ± 0.77 (do)
Acetyl-INH	1	4.01 ± 0.60 (do)	2.39 ± 0.95 (do)
	3.	7.1 ± 1.04	3.97 ± 0.38
Acetyl-INH/ Isoniazid	3	1.95 ± 0.33	0.73 ± 0.20
Acetylation (%)	3	66.11%	41.22%
Percentage of Acetylators		43.33%	56.67%

* Acetylator phenotype was determined by using acetylisoniazid/isoniazid ratio (at 3 hours) as the determinant. All values are means (± SEM) of the number of subjects indicated in parentheses.

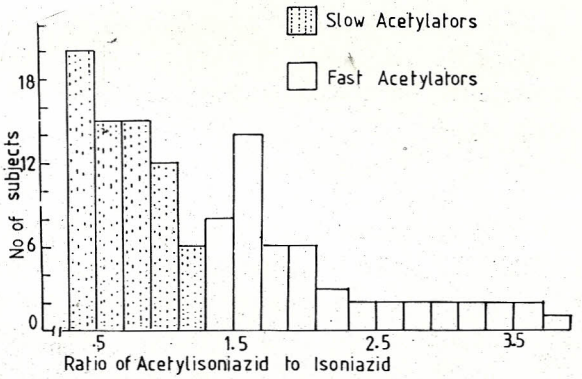

Fig. 1. Frequency distribution histogram of healthy volunteers according to ratio of acetylisoniazid/ isoniazid at 3 hours in blood.

Urinary concentrations (0 to 3 hours) of isoniazid (INH) and acetylisoniazid (Ac-INH) also differentiated the fast and slow acetylators (Table 2, Fig.2). The frequency distribution histogram (Fig.2) showed that acetylation percentage (0 to 3 hours urine) values served for the distinction of fast and slow acetylators, having 50 % as the antimode (Fig.2). The acetylisoniazid/isoniazid (Ac- INH/INH) ratio (0 to 3 hours Urine) also gave similar results of volunteers classification as slow and fast acetylators, thus confirming the above results.

Table 2

Urinary Excretion of Isoniazid and Acetylisoniazid and Percentage of Fast and Slow Acetylators in Population of Punjab

	Urinary Concentrations (mg) in 0 to 3 hours urine	
Acetylator Phenotype*	Fast Acetylators (n = 52)	Slow Acetylators (n = 68)
Isoniazid	30.60 ± 3.79	48.70 ± 5.58
Acetylisoniazid	47.92 ± 6.19	21.89 ± 2.37
Acetyl-INH/INH Ratio	1.56 ± 0.47	0.45 ± 0.42
Total Concentration (INH+Acetyl-INH)	78.52 ± 4.1	70.59 ± 3.92
Acetylation (%)	60.75 ± 4.21%	31.01 ± 3.2%
Percentage of Acetylators	43.33%	56.67%

* Acetylator phenotype was determined by using acetyl- INH/ INH ratio and acetylation percentage. All values are means (± SEM) of the number of subjects indicated in parentheses.

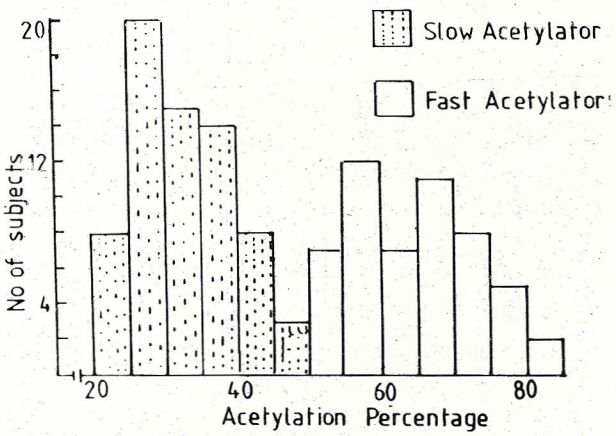

Fig. 2. Frequency distribution histogram of healthy Volunteers according to acetylation percentage of isoniazid in zero to 3 hours urine.

Among the 60 tuberculosis patients 41.67% are fast (n = 25) and 58.33% slow (n = 35) acetylators. (Table 3, Fig.3). These results closely resemble the phenotyping results of group A.

Table 3

Plasma Concentrations of Sulphamethazine and Acetyl-Sulphamethazine at 6 hours after Drug Administration and Percentage of Slow and Fast Acetylators in Tuberculosis Patients

Mean Plasma Concentrations (μg/ml)		
Acetylator Phenotype*	Fast Acetylators (n = 25)	Slow Acetylators
Free Drug (Sulphamethazine)	22.49 ± 3.24	42.87 ± 4.10
Acetylated Drug (Acetylsulphamethazine)	25.96 ± 2.95	10.37 ± 1.78
Total Drug (Free + Acetylated)	48.45 ± 3.76	53.24 ± 3.45
Acetylation (%)	53.55 ± 2.81	19.48 ± 3.01
Percentage of Acetylators	41.67%	58.33%

* Acetylator phenotype of tuberculosis patients was determined by using Plasma Acetylation Percentage (at 6 hours). All values are means (± SEM) of the number of subjects indicated in parentheses.

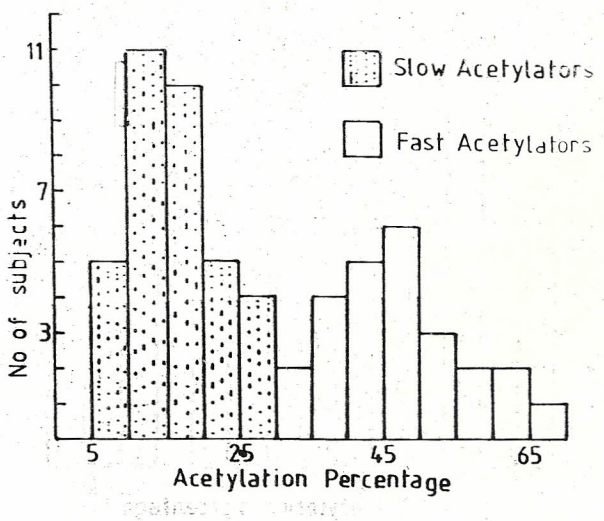

Fig. 3. Frequency distribution histogram of tuberculosis patients according to acetylation percentage of sulphamethazine at 6 hours in blood.

Collectively among 180 voluteers of group A and B, 42.7 % are fast (n =77) and 57.27 % are slow (n = 103) acetylators.

DISCUSSION

Many drugs are acetylated by liver N-acetyltransferase [8]. Different studies showed that rate of acetylation is genetically controlled giving fast and slow acetylators[1,2,5]. Present investigations of acetylator phenotyping supported the above findings about the presence of two acetylator phenotypes.

These phenotyping results of population of Punjab showed that 42.78 % are fast and 57.22 % are slow acetylators among said population. These results closely resemble with studies in Indian Population [16], according to which 58 % are slow and 42 % fast acetylators. This may be argued that Punjabi Population is related ethnically and geographically with Indian Population, due to which there is similar distribution of acetylator phenotype, because it has already been reported that ethnic and geographic distribution influence the distribution of acetylator phenotype[14,15]. Another supporting point to above assumption is that mixed population was used, which contained volunteers from families both local to Punjab (Pakistan) and migrated from India.

It is reported that method of acetylator phenotyping influences the results [3,23]. In present study three techniques of acetylator phenotyping were used simultaneously and results obtained from all of them were similar, which enhanced the reliability of these results. In group B, SMZ was used as a test drug, the results of this group are also in accordance with those in goup A, which further increases the validity of our results in both groups. Present results are in confirmation with the previous study [1], that disease (tuberculosis) has no effect on the acetylation potential because group A and B gave similar results. The transfer of acetylation potential is genetically controlled[2], which further supports the above hypothesis that disease has no effect on acetylator status.

The inclusion of volunteers having blood relation was prevented in the population study as acetylator status is genetically determined [2,15], which minimized the repetition and possible error in results. These results of acetylator phenotyping suggest that as Pakistani (Punjabi) population is divided into slow and fast acetylators, the therapy for various diseases (like tuberculosis) should be conducted according to the acetylator status of patients which may result to maximum therapeutic efficacy with minimum toxicity.

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