

# DETERMINATION OF ACETYLATOR PHENOTYPE AND INFLUENCE OF GENDER ON ACETYLATION IN A SOUTH INDIAN POPULATION

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## ABSTRACT

This study determined Acetylator status in a South Indian population and influence of gender on Acetylation using Dapsone as probe drug. The study included 96 healthy male and female volunteers. After an overnight fast, each volunteer was orally administered 100mg of Dapsone. Dapsone and its metabolite (monoacetyl dapsone) in plasma samples were estimated by HPLC. The frequency of slow acetylators was 50.00 %, the frequency of rapid acetylators was 43.75% and that of intermediate acetylators was 6.25%. The mean metabolic ratio of monoacetyl dapsone to dapsone was significantly higher in females ( $0.41 \pm 0.04$ ) than males ( $0.30 \pm 0.02$ ) with 95% confidence interval of proportions. There is a difference in Acetylation status of male and female volunteers, with preponderance of rapid acetylators in female population. It was observed that the Acetylator status of South Indians resembles that of Caucasians and Blacks (Negros).

**Keywords :** Phenotyping, Acetylation, Polymorphism, Monoacetyldapsone, Dapsone.

## 1.Introduction

Interindividual variation in response to drugs, both therapeutic and toxic, is well recognized. One of the best known examples of genetic sources of variability is N-acetylation polymorphism (Weber & Hein, 1985). Acetylation exhibits a genetically controlled bimodal distribution within any given population. Individuals can be phenotyped as either slow or rapid acetylators using a test drug. Polymorphic N-acetylation has been linked to variations in drug response, susceptibility to adverse reactions and an increased incidence of certain spontaneous disorders including cancer (Weber & Hein, 1985). A knowledge of incidence of phenotypes may provide a valuable contribution to the institution of more rational and successful therapy. Routinely determining human acetylator phenotype status might be helpful in adjusting and modifying dosage regimen. Gender-related differences in pharmacokinetics have frequently been considered as potentially important determinants for the clinical effectiveness of drug therapy (Meibohm, 2002). Dapsone has been used for the treatment of leprosy, chloroquine-resistant malaria and dermatitis herpetiformis (Weber & Hein, 1985). It is also used as a test drug for determination of the acetylator phenotype with results comparable with those obtained using isoniazid and sulphamethazine (sulphadimidine) (Irshaid, 1991). Dapsone is monoacetylated in man by the same enzyme system that acetylates isoniazid and sulphamethazine (Gelber, 1971). The genetic polymorphism of human

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N-acetyltransferase 2 (NAT2) divides the human population into groups with rapid, intermediate and slow acetylator status (Brocvielle, 2003). Monoacetyldapsone/dapsone ratios of  $< 0.30$  indicate slow acetylation and those of  $> 0.30$ , rapid acetylation (Philip, 1989). Using Dapsone as a marker, this study investigates acetylator phenotype in a South Indian population and influence of gender on acetylation.

## 2.Materials and Methods

Ninety six (44 males and 52 females who ranged in age from 18-36 years and weighing between 45-73 kg) unrelated healthy males and females have participated in the study. They were either students or employees of Kakatiya University, Warangal, Andhra Pradesh, South India. The volunteers were briefed about the study and informed written consent was obtained from each subject. All participants had no history of serious medical illnesses, were normal on physical examination and had normal serum creatinine, alanine amino transferase (ALT) and normal glucose-6-phosphate dehydrogenase activity. Subjects who are smokers, alcohol drinkers, pregnant women, subjects with a history of allergy or those taking medications were excluded. The study was approved by the local University Ethics Committee.

After an overnight fast, each subject received a single oral 100 mg dose of Dapsone. Drinking of caffeine containing beverages was not allowed throughout the study period. A blood sample (7 ml) was obtained by venepuncture into a heparinized tube 3 hr after drug intake. Plasma was separated immediately by

centrifugation at 3000 rev min<sup>-1</sup> and stored frozen at -20° C pending analysis (Irshaid, 1991).

Dapsone and its metabolite monoacetyldapsone concentrations in plasma were measured by modification of the reported HPLC method (Philip, 1984). The HPLC system (Shimadzu, Japan) consisted of UV-visible spectrophotometric detector. The mobile phase consisted of water: acetonitrile: acetic acid (100:40:2.5v/v/v) with a flow rate of 1.5ml/min. The column used was Hicrom C-18 (stainless steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres) and the eluent was monitored at 280 nm. To 0.5ml of plasma, 100µl of metronidazole (20µg/ml) was added as internal standard and alkalized with 0.5 ml of 0.2% NaOH and vortexed for two minutes, then extracted with 8 ml of diethyl ether by vortexing and centrifuged at 800 rpm for 10 minutes. The supernatant is evaporated to dryness and reconstituted with 50µl of acetonitrile. 20µl of the reconstituted sample is spiked onto the column.

### Statistical Analysis

Results are presented as means ± s.e. mean. Differences between group means were assessed by the unpaired Student's t-test and considered significant when the P value was <0.05. Linear correlation between the acetylation ratio and Dapsone or Monoacetyldapsone concentration was tested by simple regression analysis.

### Materials

Dapsone powder (Burrough's Wellcome, Mumbai), Dapsone tablets 100mg (Burrough's Wellcome, Mumbai), Metronidazole powder (Aristo Pharmaceuticals, Mumbai), Methanol (HPLC grade) (Qualigens Fine chemicals, Mumbai), Acetonitrile (HPLC grade) (Qualigens Fine chemicals, Mumbai), Acetic acid (HPLC grade) (Merck, Mumbai), Diethyl ether (s.d.Fine Chemicals Ltd, Mumbai) and Sodium hydroxide (Gravimetric Purity) (Merck, Mumbai) were used as received.

### 3.Results and Discussion

In the case of males, out of total 44, twenty five (56.82%) are slow acetylators, sixteen (36.36%) are rapid acetylators and three (6.81%) are intermediate acetylators. Out of total 52 females, twenty three (44.23%) are slow acetylators, twenty six (50%) are rapid acetylators and three (5.77%) are intermediate acetylators. On the whole, the distribution of acetylator phenotypes in the subjects is: forty eight (50%) slow acetylators, forty two (43.75%) rapid acetylators and six (6.25%) intermediate acetylators. (Table-1).

From the previous studies on NAT2 polymorphism, similar results were reported: 50-60% of Caucasians, Blacks, South Indians and Mexicans were found to be slow acetylators (Brocvielle, 2003), a study conducted in South Indians reported the results of NAT2 genotyping - slow acetylators were found to be predominant.(Anitha, 2003).

Also, data obtained from earlier studies carried out in different populations or ethnic groups, indicate that the overall proportion of slow acetylators among South Indian population is very similar to the proportions found among all Caucasian groups, whether they be in United states, Germany, Finland, Britain, Sweden, Czechoslovakia, or Canada. Studies carried out among Negroes in Sudan, United States, East Africa and Nigeria suggest that the proportion of slow acetylators in this racial group is also similar to those among South Indians and Caucasians (Ellard, 1976). Thus, the present investigation indicates that the South Indians resemble Caucasians and Blacks (Negros) in acetylation.

Statistical analysis was performed using Unpaired t-test (two-tailed). There was a significant difference (P=0.026) in frequency distribution of slow and rapid acetylator phenotypes between male and female volunteers.

The mean metabolic ratio of monoacetyldapsone to dapsone was significantly higher in females (0.41±0.04) than males (0.30±0.02) (Table- 2) with 95% confidence interval of proportions.

The frequency distribution profiles for plasma monoacetyldapsone to dapsone ratios are shown in figure (Fig.1). The histograms are visually suggestive of a bimodal distribution.

As for the influence of gender on acetylation (Fig.2), statistically significant difference was detected between slow and fast acetylators in terms of gender in the present study.

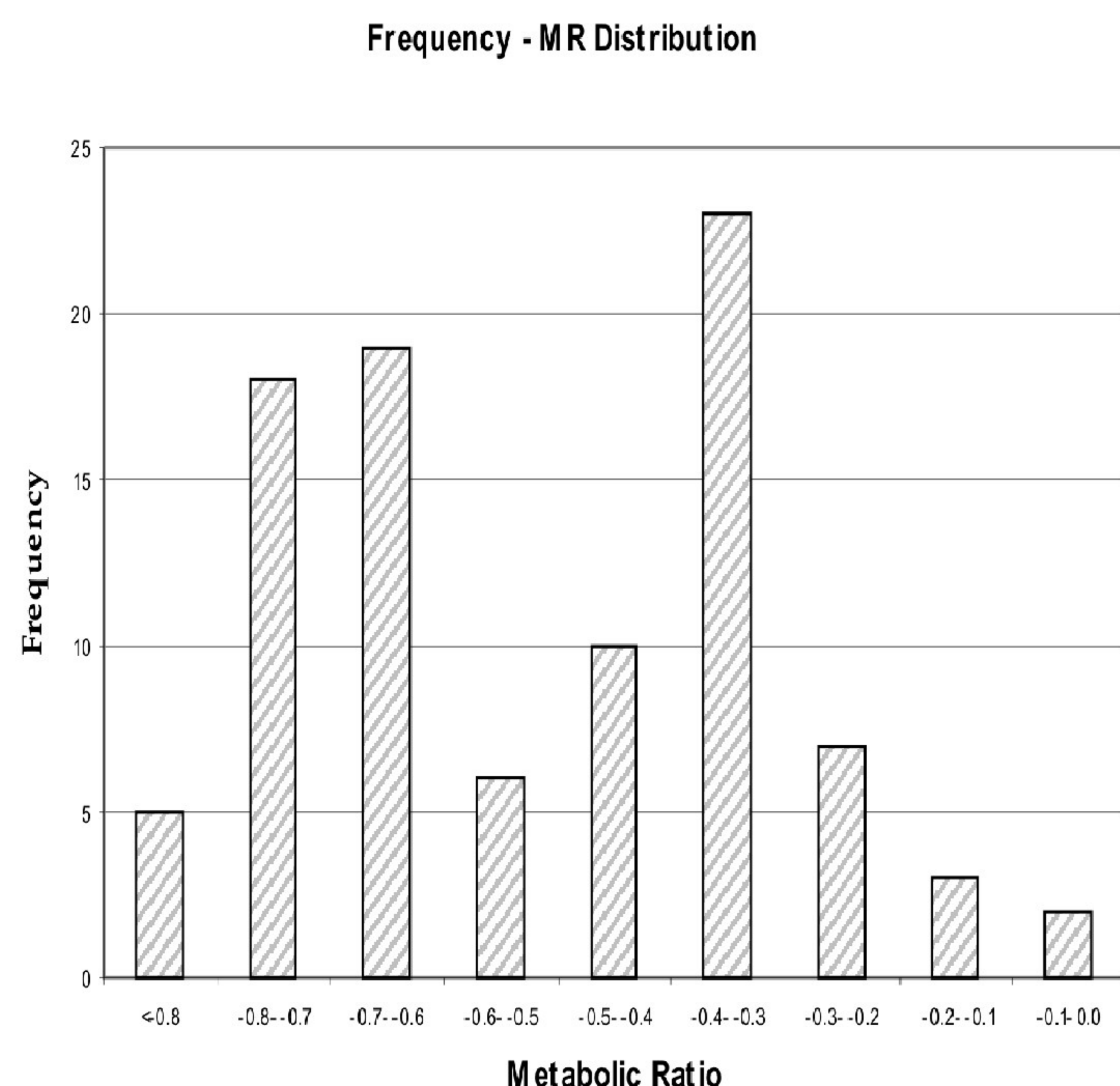
### 4.Conclusion

There is a higher incidence of slow acetylators in the study population. These results are in agreement with previous similar studies with other probe drug (s) in South Indians, where proportion of slow acetylators predominates rapid acetylators. There is a significant difference in acetylation status of male and female volunteers, with preponderance of rapid acetylators in female population. And, the present study reveals that the South Indians resemble Caucasians and Blacks (Negros) in acetylation. This approach of assessing the phenotypic measures of drug metabolizing activity helps

to predict adverse reactions and is also applicable to other drugs with metabolism based adverse effects.

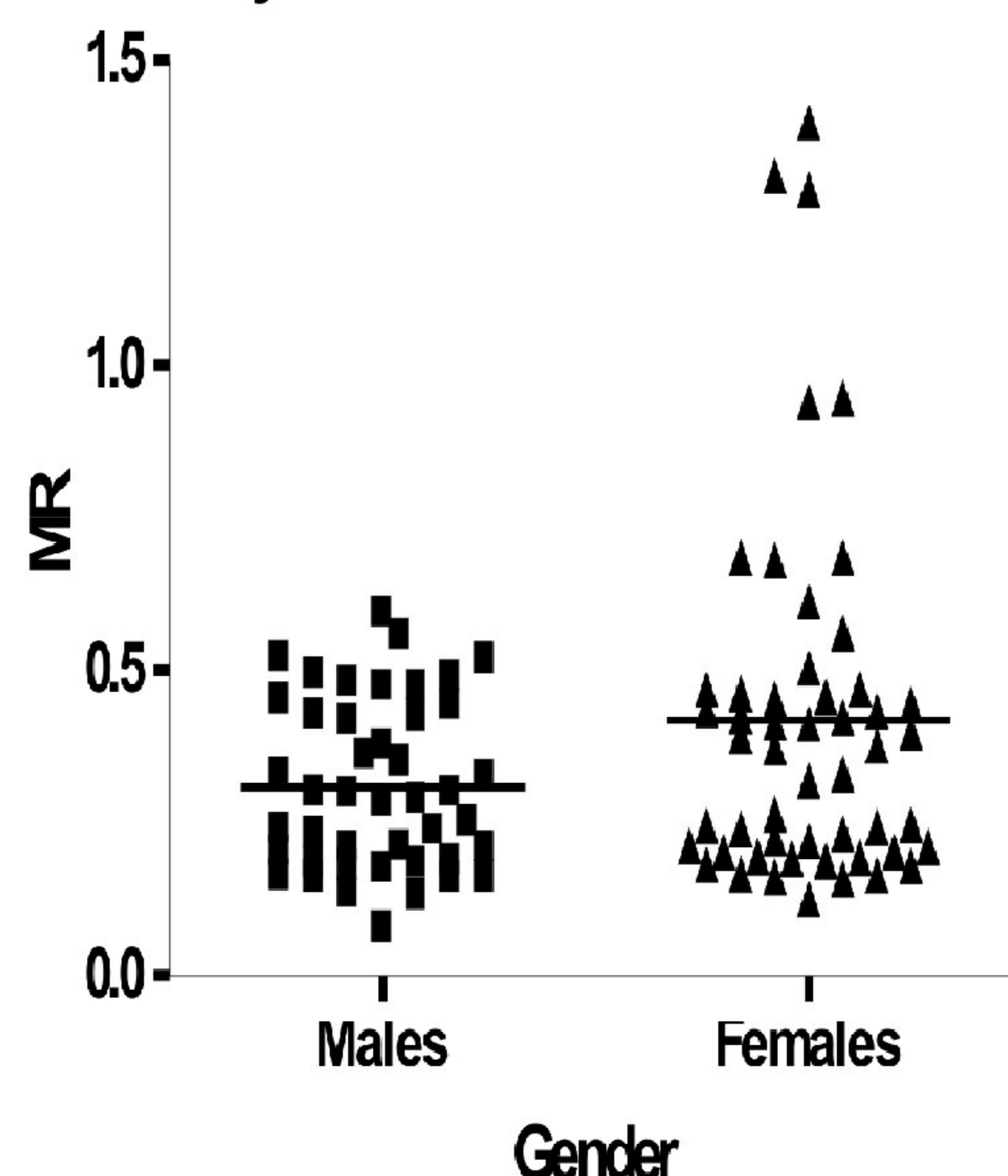
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**Fig.1** Frequency distribution of the plasma monoacetyldapsone/dapsone acetylation ratio in 96 volunteers.

## Dapsone Acetylation Status in Indian Males & Females



**Fig.2.** Acetylation status in males and females

**Table-1** Distribution of Acetylator phenotypes observed in 96 healthy volunteers.

(No. of volunteers is shown in parentheses)

| Gender | No. of volunteers | Percentage(%) of Acetylator Phenotypes |           |              |
|--------|-------------------|--|-----------|--------------|
|        |                   | Slow                                   | Rapid     | Intermediate |
| Male   | 44                | 56.82(25)                              | 36.36(16) | 6.81(3)      |
| Female | 52                | 44.23(23)                              | 50.00(26) | 5.77(3)      |
| Total  | 96                | 50.00(48)                              | 43.75(42) | 6.25(6)      |

**Table-2** Mean metabolic ratios of monoacetyldapsone to dapsone

| Gender | Concentration of drug(Mean $\pm$ SEM) | Concentration of metabolite(Mean $\pm$ SEM) | Metabolic ratio (Mean $\pm$ SEM) |
|--------|---------------------------------------|---|----------------------------------|
| Male   | 0.9894 $\pm$ 0.0926                   | 0.2922 $\pm$ 0.03207                        | 0.3098 $\pm$ 0.02075             |
| Female | 0.9137 $\pm$ 0.08438                  | 0.3274 $\pm$ 0.03092                        | 0.4197 $\pm$ 0.04106             |

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