DISCUSSION

The present study detected 16 subjects with a decreased *CYP2D6* activity giving the poor metaboliser frequency of 3.52% in South Indian population. This result suggests that, overall, the South Indian population may have distribution characteristics intermediate between Caucasians (5-10%) and other Asian population (0-1%). The incidence of PM in Kerala (4.8%), Karnataka (4%), and Tamil Nadu (3.6%) population is more than that observed in Andhra subjects (1.8%).

Based upon the genetic characteristics, the people of India have been broadly classified into six main ethnic groups: Caucasoid Aryans, Caucasoid Dravidians, Australoids, Negroids, Western Brachycephals and Mongoloids. The Australoids, Negroids, Western Brachycephals and Mongoloids are the tribal populations of India[198]. The non-tribal population of India consists mainly of Caucasoid Aryans in North India and Caucasoid Dravidians in South India[198, 201]. The studies in South Indian population shows that the Dravidian population has a higher frequency of PM phenotype when compared to other Oriental races.

Andhra Pradesh consists of three distinct regions. (i) Coastal region generally called Andhra, (ii) the interior region known as Rayalaseema and (iii) Telengana region, consisting of the capital Hyderabad and adjoining districts. The present study population is from Andhra region. The earliest mention of the Andhras is said to be in Aitereya Brahmana (2000 BC). It indicates that Andhras originally an Aryan race, living in North India, migrated to the south of Vindhyas and later mixed with non-

Aryan stocks[199]. The low frequency of PM in Andhra Pradesh population can be compared with the other studies conducted in populations of migrated Indo-Aryan races. The subjects of Bombay[103] and Sinhalese from Sri Lanka[104] also showed a low prevalence of poor metaboliser phenotype (0-2%) like Andhra population. However the mean metabolic ratio or frequency distribution histograms of Andhra subjects were not significantly different from other South Indian states. Further studies including the genotyping of the subjects may give a clearer picture about the genetic polymorphism in this region.

Table-9 shows all the reported studies of *CYP2D6* phenotyping in South Asian population. It shows that subjects of pure Dravidian origin have a higher frequency of poor metabolisers compared to the migrated Aryan subjects. The Aryan subjects of India and Sinhalese in Sri Lanka, show less frequency (0-3%) than the Dravidian subjects from South India (3.2-4.8%). However, the frequency of poor metabolisers reported with all the South Indian population is within the confidence interval (95%) reported with other South Asian reports.

In Sri Lanka, the studies have reported only with the Sinhalese population from Colombo. However, the other major ethnic group in Sri Lanka is the Tamils originated in South India[104]. Since the Tamil population is ethnically similar to the present study population from South India, the present study may also be relevant to the Tamil subjects in Sri Lanka.

There is a marked rightward shift in the frequency distribution histogram of the metabolic ratio of South Indian population compared to Caucasian population[38, 117] ($P<0.001$). This shift is comparatively less when compared to the Chinese population[143]. However the frequency distribution of the present study population is comparable with that reported in North Indian population[8] ($P>0.05$). Horai *et al.*[146] reported that the frequency distribution curve for metoprolol metabolic phenotype in Chinese population was skewed to the right compared with that in Japanese population. The findings are further substantiated by another study comparing metabolic ratio of metoprolol between Chinese, Japanese and Korean population[145]. Chinese EM showed a lower capacity to metabolise metoprolol to α-hydroxy metoprolol compared with Korean or Japanese extensive metabolisers. This shift in metabolic ratio in Chinese population was observed with debrisoquine also when compared to Swedish[123] and Taiwanese[39] population.

A similar inter-ethnic difference in the distribution histograms of debrisoquine and metoprolol extensive metabolisers has also been observed between two non-Oriental (British and Nigerian) populations[138]. Kalow[97] compared the distribution capacity of *CYP2D6* in four human populations from different locations (China, Africa, Sweden and Spain). The average enzyme activities of both Africans and Chinese are lower than in both European populations. The main *CYP2D6* difference between Spaniards and Swedes is a more frequent gene duplication in the former, leading to

more cases with very high enzyme activity. Environmental factors may modulate genetic expression, which may give rise to differences in the antimode of the metabolic ratio between ethnic groups[58]. The differences between White subjects and South Indian subjects in life style, dietary habits and/or occupation might have influenced the CYP2D6 isoenzyme in South Indian population. This may be the reason for the difference in the mean metabolic ratios of these ethnic groups.

However, the mean metabolic ratio of extensive metabolisers is significantly different between the present study population and another South Indian study from Hyderabad city population[9] ($P<0.001$). The very low mean metabolic ratio observed in that study (0.021) was significantly different compared to the North Indian[8] population (0.088) also. The authors of the Hyderabad population study could not explain the reason behind this marked shift. Moreover, there was not much difference in the poor metaboliser frequency between the Hyderabad (3.2%) and North Indian population (3%). The population selected for that study was only from the Hyderabad City. Since Hyderabad is a metropolitan city, chances of genetic mixing are more in that population. Subjects from different parts of India including the tribes of India may be present in the city population. This genetic mix may be the reason for the shift in the histogram of that study. However, this difference cannot be authentically explained without a genetic analysis. The result obtained in the preset study from the four different states was not significantly different and is comparable to the North Indian study.

Well-established differences exist between Caucasians and Orientals including the responsiveness to ethanol involving differences in both alcohol and aldehyde dehydrogenases, and also the different distribution of slow and fast acetylators of N-acetyltransferase. Both anecdotal and experimental reports indicate that drug responsiveness may differ substantially between ethnic groups such as Caucasians and Orientals, so that there are significant differences in dosage requirements[6].

It has been recognized that although the poor metaboliser frequency is significantly lower in Orientals, mean *CYP2D6* activity is also lower in Orientals than in Caucasians. Many Orientals consequently have a reduced ability to metabolize antidepressant and neuroleptic drugs that are substrates for *CYP2D6*. Indeed many antidepressants are prescribed at lower doses in oriental populations because of the higher mean metabolic ratio for *CYP2D6* substrates and the lower associated plasma clearance time[6].

An interesting point worth considering with regard to geographic polymorphism is the possibility of genetic regulatory mechanisms at the population level. That is, allelic frequencies giving a high 'genetic load' at one locus are often associated with those giving low genetic loads at other loci[200]. This may be the reason for the low *CYP2D6* and high *CYP2C19* PM frequency in Chinese population compared to the high *CYP2D6* PM and low *CYP2C19* PM prevalence in Caucasian population.

DNA marker studies reported that Indian and European populations have common Caucasoid ancestor and are genetically distinct from those of Oriental population[105]. However, the phenotypic studies of *CYP2D6* activity in India shows that the Indian population is a separate group with the enzyme activity in between the Caucasian and Oriental subjects. The study of *CYP2C19* polymorphism in North Indian subjects (11%) also indicated that cytochrome P-450 activity in Indian population is different from other ethnic groups[106]. However, no report is available about the frequency of *CYP2C19* polymorphism in South India.

Considering the international nature of drug development and use, and the multiracial nature of the populations of many countries, the potential for genetically determined interethnic differences in drug responsiveness due to drug dispositional factors should be given increased recognition[4].

The Hardy-Weinberg principle states that in a large population, barring disturbances by outside influences, the relative proportions of the possible genotypes as a particular locus remains constant from one generation to the next. The principle as applied to human population is probably only an approximation because the principle is only absolutely true under certain very specific conditions, and probably for any particular trait in man, all these conditions are rarely satisfied. The Hardy-Weinberg principle is only true for a large population where there is a) a constant

rate of mutation, b) random mating, c) no selection for as particular genotype and d) no migration[200].

None of the various factors studied could influence the metabolism of dextromethorphan. The age, sex and body mass index did not appear to be related to metabolic ratio in South Indian population. This is in accordance with the various reported studies[57, 58, 65, 66]. Alcohol consumption and smoking did not significantly change the metabolic status of the study population. Straka *et al.*[56] reported a significant association between metabolic ratio and smoking status in White subjects. However, the same study could not find an association between metabolic ratio and smoking status in Hmong population. Other population studies also observed that smoking is not significantly altering the *CYP2D6* activity[57, 58]. Since *CYP2D6* is non-inducible by environmental factors[16], smoking may not induce its activity significantly.

Unlike Western population none of our female participants were smokers or alcoholics. This may be because of the strict social restrictions in this region. In males chronic alcoholism was one of the exclusion criteria, so that only occasional social drinkers participated in the study. Their alcohol consumption was less than 20 units per month. Similarly chronic smokers also were excluded from the study population. Moreover, since most of our study subjects were of undergraduate students, history of their smoking or alcohol usage was also less. However, it is

difficult to derive a conclusion with the present study due to the small number smokers in the study population.

In South India, some people follow vegetarian lifestyle as a part of their religious custom over many generations. For examples most of the Brahmin families are vegetarians. In our study population, 53 subjects were vegetarians and we compared the metabolic ratios of the vegetarians with the non-vegetarians. There was no significant difference in the metabolic pattern of vegetarian and non-vegetarian volunteers. This again suggests that, *CYP2D6* activity cannot be altered like most of the other enzymes.

Christian and Muslim religions are coming under the minority groups in India. Though there was no PM among Muslim volunteers, the metabolic ratio was not significantly different between the three religious groups. However, all these subjects are from same ethnic origin. This may be the reason for the lack of influence of the religion in the *CYP2D6* activity. Moreover, the lifestyle also is not much different between different religious grooups in South India. So this parameter may not be a significant factor in the population studies in a place like South India.

It has been proven that pregnancy can influence the *CYP2D6* enzyme activity, and hence pregnancy was an exclusion criteria while selecting the female subjects[68, 69].

However the menstrual cycle phase has not been considered in female subjects during study because of the lack of influence of menstrual cycle phase on dextromethorphan metabolic ratio[63].

Dextromethorphan was chosen as the probe because its oxidative polymorphism is well documented and cosegregates with that of debrisoquine and sparteine[73]. Its relatively innocuous pharmacodynamic profile and easy availability in India compared with debrisoquine was also considered while selecting dextromethorphan as the probe drug.

Antimode (0.3) used in the present study is reported with the Caucasian population[73]. In the reported North Indian and South Indian studies also there was a separation at 0.3. However, this antimode may not be suited for the Indian population. In Sri Lanka, the *CYP2D6* phenotyping using debrisoquine showed that the Caucasian antimode of 12.6 could not classify the population into different phenotypes. Therefore, in the present study also there are chances of misinterpretation only by phenotyping. Some of the subjects with a borderline metabolic ratio may be heterozygous extensive metabolisers. A detailed genetic analysis may give a more clear picture about this population.

The bimodality in the distribution of MR analysed by 3 method (histogram, probit plot and NTV plot). Visual inspection in the histogram, a non-linearity of the probit

plot and negative values in NTV plot showed a bimodal distribution in the study population. However, histogram and probit plot were easier method in assigning the phenotype. Yue et al. also observed that histogram is more preferable over a NTV plot in determining phenotype[202]. Sharp break point in a probit plot also has been used to determine the antimode[71]. However, in the present study population this method will not change the frequency of poor metabolisers.

Polypharmacy and over-the counter drug purchase is very common in developing countries such as India and Sri Lanka. Since CYP2D6 is responsible for the metabolism of most of the commonly used drugs, this may result in severe drug interactions. Routine phenotyping or genotyping may not be practical in developing countries due to economic reasons. However, monitoring of CYP2D6 enzyme activity is important for the patients who report adverse reactions with normal dose of the drugs. This may help the physicians in individualization of the therapy especially for diseases requiring long term therapy like depression and hypertension.

Although the potential importance of genetic variability in drug response is generally acknowledged in academic circles, the pharmaceutical industry and the drug regulatory authorities, this is not yet the case in general practice and, indeed, in many clinical pharmacology departments. A greater awareness of this is urgently needed. Since many of the drug metabolized by *CYP2D6* are CNS active agents with narrow therapeutic indices, over treatment and accumulation can give rise to

symptoms similar to those of the disease itself[2]. Doctors need to be aware of whether a drug they are prescribing is subjected to pharmacogenetic variability and its importance and potential drug interactions. Prescribing advice should highlight the possibility of drug interactions when multiple drugs are prescribed concomitantly[159].

Genotyping is preferred to phenotyping in determining enzyme activity to avoid the influence of other drugs and disease states[28]. However, in healthy subjects, phenotyping value usually correlates with the genotyping data[203]. Since genotyping is a more costly procedure and rarely available in developing countries like India, phenotyping is preferred for routine purposes. Moreover, phenotyping is the only way to understand the action of the gene in the organism and remains essential for investigating the functional implications of genomic DNA changes[10]. Serious ethical questions are raised by the increasing routine storage of DNA samples and the genetic information might be entered into computerized database. Such storage presents novel issues related to autonomy, privacy and informed consent that warrant clarification, discussion and immediate action. It has been argued that genetic information is more vulnerable to violation of privacy because it contains an 'individual's probabilistic "future diary"'[204]. However, for patients undergoing concomitant therapy with the drugs, which can affect on *CYP2D6* activity, genotyping should be used[11].

In the present study period, we have tried all the possible ways to carry out the genotyping of *CYP2D6* in South Indian population. However, we could not succeed in our attempt due to the lack facilities available for genotyping.

Our increasing knowledge of the mechanisms of drug action, the identification of new drug targets and the understanding of genetic factors that determine our response to drugs may allow us to design drugs that are specifically targeted towards particular populations or that avoid genetic variability in therapeutic response[159]. Since up to 5% of the Indian population is deficient in CYP2D6 activity, caution should be taken to avoid adverse drug reactions and individualization of drug treatment may be considered.

Our study is the first study to report the CYP2D6 activity in all the four South Indian states. This study has covered subjects from all the areas of South India because the subjects who participated were from the different areas of the states studied.

Since the South Indian population has migrated widely to different parts of the world, these results may serve as a basis to test relationship between drug oxidation polymorphism and drug induced adverse reactions or diseases of unknown etiology in this population.