

RESULTS

Volunteer characteristics:

In the present study, 454 volunteers participated. Their age ranged from 16 to 55 years. Among the participants, 309 subjects were males (aged 16-55 years) and 145 were females (aged 16-45 years). The mean (SD) age of male and female participants was 23.45 (6.15) and 21.98 (3.82) respectively. This difference was not statistically significant. Maximum number of volunteers (420) were in the age group of 18-30 years. Only 26 subjects were in between 31-50 years of age and 3 subjects were above 50 years. Five participants were less than 18 years of age (4 males and 1 female).

The body mass index (BMI) of participants ranged from 12.74 to 38.22. Its mean (SD) BMI of male and female volunteers were 20.94 (3.07) and 20.32 (2.92) respectively.

General physical examination showed that all volunteers were in good health and their blood pressure, respiratory rate and temperature were in the normal range. None of the participants were taking any drugs, (including Ayurvedic drugs) which is known to influence the metabolism of dextromethorphan. About 25 subjects reported previous history of allergy including dust, sulphonamides and penicillin.

Most of the subjects were Hindus (364) and the number of Christian (73) and Muslim (17) participants were comparatively less. Only few volunteers were

occasional users of alcohol (63) or tobacco (37). Fifty three subjects were pure vegetarians.

Table-6 shows the comparison of demographic characteristics of 454 subjects from the 4 South Indian states. The age and BMI were not statistically significant among the four states.

In **Kerala**, the volunteers were within the age group of 17 and 44 years. The mean (SD) age of males (n=66) was 24.12(4.86) and for females (n=38) it was 21.56(1.47) and it showed a statistically significant difference (P=0.003). The mean (SD) BMI of male volunteers {21.15 (2.54)} was significantly higher than the female volunteers {20.06(2.58)} (P=0.045).

In **Karnataka**, the age group of volunteers ranged in between 16 and 45 years. The least percentage of female participants (17%) in the present study was from Karnataka. The mean age or BMI was not statistically significant between the male and female participants. The demographic characters of **Andhra Pradesh** and **Tamil Nadu** volunteers were similar to the Karnataka subjects. Compared to all other states, the proportion of vegetarian participants was more from Andhra Pradesh (24.32%). Similarly, the percentage of female participants was maximum (36.7%) in Tamil Nadu.

Metabolism of dextromethorphan:

The MR ranged from 0.00236 to 5.678 for males and 0.0032 to 7.295 for females. The mean (SD) metabolic ratio of males and females were 0.11 (0.51) and 0.14 (0.67) respectively ($P>0.05$).

In MR, there was a gap between 0.246 and 0.325. The reported antimode of 0.3 is in-between these values and the subjects were classified with respect to their MR. Sixteen subjects had metabolic ratio higher than the antimode and were classified as PM. The MR obtained were subjected to the Kolmogorov-Smirnov test, and the data failed normality test (Kolmogorov-Smirnov distance (KS) = 0.42, at $P<0.0001$).

There was bimodality in the frequency distribution histogram (Figure-11), non-linearity in the probit plot (Figure-12) and negative NTV values in the NTV plot (Figure-13). All the three figures clearly demonstrated that the MR of South Indian population was not following a normal distribution and 16 subjects had a different phenotype compared to other 438 subjects. Hence the present study showed that the frequency of PM of *CYP2D6* was 3.52% (95% confidence interval of 2.03-5.66%) in South Indian subjects. Table-7a compares the MR and Table-7b demonstrates the percentage of EM and PM subjects among the 4 South Indian states.

Urinary 8-hour excretion of dextromethorphan and its metabolite dextrophan showed large inter-individual differences (Table 8). The urinary output of DM and DT for EM and PM are given in Table-8. The unchanged DM excretion ranged from 0.23 μ mol to 19.44 μ mol and the excretion of DT ranged from 0.06 μ mol to 71.9 μ mol. EMs excreted approximately 29.8% of administered dose as DT but PM excreted only about 2.68% as DT. PM excreted 3.9 fold more DM than EM.

Among the 16 PMs, 9 subjects were males and 7 were females. The mean (SD) MR of male and female PM were 2.35(2.5) and 2.27(2.5) respectively and the difference was not statistically significant. Table-8 demonstrates the comparison of PM and EM subjects. No significant difference was observed in any of the demographic parameters between PM and EM subjects.

Figures 14-25 shows the frequency distribution histogram, the probit plot and the NTV plot of Kerala (Figure 14-16), Karnataka (Figure 17-19), Andhra Pradesh (Figure 20-22) and Tamil Nadu (Figure 23-25). The metabolic ratio of all the four states failed the normality test and showed a polymorphism in the dextromethorphan metabolism. There was no significant difference in the mean MR in-between the four South Indian states. The mean MR for the four states were in the following order: Karnataka (0.23 \pm 0.9) > Tamil Nadu (0.11 \pm 0.57) > Kerala (0.11 \pm 0.32) > Andhra Pradesh (0.06 \pm 0.09). The KS also followed the same pattern

with a maximum distance of 0.47 with Karnataka and least with Andhra Pradesh of 0.28.

In **Kerala**, the MR ranged from 0.0034 to 3.149. The frequency distribution histogram (Figure-14), the probit plot (Figure-15) and the NTV plot (Figure-16) show that the MR of Kerala population was not following a normal distribution ($KS = 0.37$, at $p < 0.0001$). The PM frequency of Kerala population was 4.8% with a 95% confidence interval of 1.6-10.9%. Among the 5 PM subjects, 4 were males and only one was female. For EM, the MR ranged from 0.0034 to 0.193 and for the 5 PM subjects the MR ranged from 0.325 to 3.149. Figures 14 and 15 show that there are 3 participants with very low MR and are separated from the other EM subjects. Their MR ranged from 0.0034 to 0.0038 and these subjects may be classified as UEM. However, no clear criteria have been reported to classify UEM by phenotyping only.

In **Karnataka**, the MR of the volunteers ranged from 0.0054 to 7.295. Figure-17 shows the frequency distribution histogram, figure-18 is the probit plot and the figure-19 is the NTV plot of the 100 Karnataka subjects phenotyped. All the three figures demonstrate that the MR of Karnataka population was not following a normal distribution ($KS = 0.473$, at $P < 0.0001$). The PM frequency of Karnataka population was 4% with a 95% confidence interval of 1.1 - 9.9%. There were 3 males and one female among the 4 PM subjects. For EM, the MR ranged from

0.0054 to 0.258 and for the 4 PM subjects the MR ranged from 1.37 to 7.295. There was no subject with MR in-between 0.258 and 1.37, in Karnataka population. The MR of PM and EM subjects were very well separated (Figure-16).

Compared to other three states, the MR of **Andhra Pradesh** population was more close to a normal distribution (Figure-20). However the MR failed the normal distribution test significantly (KS = 0.28, at $p < 0.0001$). The MR ranged from 0.0042 to 0.843. Only two subjects (1 male and 1 female) had MR higher than the anti mode so that the PM frequency of Andhra Pradesh population was 1.8% with a 95% confidence interval of 1.06 - 6.08%. Frequency distribution histogram (Figure-20) and probit plot (Figure-21) demonstrate that one subject with a very low MR (0.0042) is separated from the other EM subjects. This subject may be an UEM from Andhra Pradesh population. However, this separation is not so evident in the NTV plot (Figure-22). For other EMs, the MR ranged from 0.0062 to 0.190 and for the 2 PM subjects the MRs were 0.407 and 0.843.

The MR of **Tamil Nadu** population ranged from 0.0024 to 7.112. Figure-23 shows the frequency distribution histogram, figure-24 is the probit plot and the figure-25 is the NTV plot of the 139 Tamil Nadu subjects phenotyped. All the three figures demonstrated that the MR of Tamil Nadu population was not following a normal distribution (KS = 0.426, at $P < 0.0001$). The PM frequency of Tamil Nadu population was 3.6% with a 95% confidence interval of 1.2 - 8.2%. Unlike the

Kerala and Karnataka population, majority of the PM subjects was females (4 out of 5) in Tamil Nadu population. For EM, the MR ranged from 0.0024 to 0.246 and for the PM subjects the MR ranged from 0.455 to 7.112. The probit plot and NTV plot show that there are two subjects with very low MR and is separated from other EM subjects. However, this separation was not evident from the histogram. These two subjects may represent the UEM of Tamil population.

Based on the Hardy Weinberg equilibrium, the mutant allele occurred at a frequency of 19% and wild type occurred at a frequency of 81% in South Indian subjects. It can be predicted that around 30% population is heterozygotes. The Hardy Weinberg equilibrium of all the four states are given in Table -9.

Influence of various factors on MR

None of the volunteers reported any serious side effects. Only one subject reported adverse effect of nausea and was an extensive metaboliser. None of the factors analysed (age, sex, body mass index, religion, food habits, alcohol, and tobacco) were influenced the metabolic ratio of dextromethorphan. Age ($r = 0.139$, $P > 0.05$) and body mass index ($r = -0.017$, $P > 0.05$) showed poor correlation with the MR.

Factors like sex or food habit had not significantly influenced the MR. The mean MR of 53 vegetarian subjects was 0.118 ± 0.43 (range: 0.0032 to 3.047) and that of

401 non-vegetarian subjects was 0.123 ± 0.58 (range: 0.00236 to 7.295). This difference in MR of vegetarian and non-vegetarian was not statistically significant. Only two PM subjects were vegetarians.

Among 37 cigarette smokers, the MR ranged from 0.0112 to 0.40 {mean (SD), 0.068 (0.066)} and for 417 nonsmokers, it ranges from 0.00236 to 7.295 {mean (SD), 0.128 (0.58)}. However, the difference was not statistically significant. Only one tobacco user was identified as PM.

About 63 subjects were occasional alcohol users. Their MR ranged from 0.00378 to 5.758 {mean (SD), 0.159 (0.71)}. For non-alcohol users it was from 0.00236 to 7.295 {mean (SD), 0.113 (0.52)}. The MR of these two groups was not statistically significant. Two of the PM subjects were occasional alcohol users.

The subjects belonged to Hindu, Christian and Muslim religion (Table-6) and majority of the subjects were Hindus. The mean (SD) MRs for Hindus, Christians and Muslims were 0.13(0.61), 0.099 (0.36) and 0.06 (0.04) respectively. However the ANOVA with post-tests showed that there was no significant difference in the metabolic ratio between the religious groups. Among 16 PM subjects, 14 were Hindus (14/364) and 2 were Christians (2/73). No Muslim subject (0/17) was identified as PM in the present study.

The MR of subjects with history of various allergies was not significantly different from the remaining participants. None of the PM subjects reported with the history of allergy or recent drug treatment.

Table 6: Characteristics of the volunteers from the four South Indian states.

	KE	KA	AP	TN	Total/ Average
<i>Total subjects</i>	104	100	111	139	454
Male(%)	66(63.5)	83(83.0)	72(64.9)	88(63.3)	309(68.1)
Female(%)	38(36.5)	17(17.0)	39(35.1)	51(36.7)	145(31.9)
Age (SD)	23.2(4.1)	24.1(4.9)	23.4(6.7)	21.7(6.3)	22.9(5.5)
BMI (SD)	20.7(2.6)	20.5(3.4)	20.4(3.0)	20.8(3.1)	20.8(3.1)
Religion					
<i>Hindu (%)</i>	37(35.6)	90(90.0)	104(93.7)	133(95.68)	364(80.2)
<i>Christian(%)</i>	59(56.7)	03(03.0)	05(04.5)	06(04.3)	73(16.1)
<i>Muslim(%)</i>	08(07.7)	07(07.0)	02(01.8)	00(00.0)	17(03.7)
Alcohol users(%)*	20(19.2)	29(29.0)	03(02.7)	11(07.9)	63(13.9)
Smokers(%)	08(07.7)	19(19.0)	02(01.8)	08(05.8)	37(08.1)
Vegetarians(%)	01(00.1)	17(17.0)	08(07.2)	27(19.4)	53(11.7)
History of allergy(%)	05(04.8)	04(04.0)	06(05.4)	10(07.2)	25(05.5)
ADR	00	00	00	01	01

KE= Kerala, KA= Karnataka, AP= Andhra Pradesh TN= Tamil Nadu

BMI =Body mass index and ADR = Adverse drug reactions with the test drug.

*Occasional alcohol users

Table7 (a): Comparison of the mean metabolic ratio(SD) in the four South Indian states.

	KE	KA	AP	TN	Mean(SD)
EM	0.06(0.05)	0.057(0.03)	0.057(0.04)	0.042(0.05)	0.054(0.04)
PM	1.02(1.2)	3.206(2.34)	0.859(0.28)	2.91 (2.64)	1.997(2.25)

Values are mean (SD)

Table7 (b): Comparison of the CYP2D6 phenotypes in the four South Indian states.

	KE	KA	AP	TN	Total (%)
EM(%)	99(95.2)	96(96.0)	109(98.2)	135(96.4)	439(96.48)
PM(%)	05(04.8)	04(04.0)	02(01.8)	05(03.6)	16(03.52)

KE= Kerala, KA= Karnataka, AP= Andhra Pradesh and TN= Tamil Nadu

Values are total (%)

Table 8: Urinary output (0-8 hours) and dose recovery of DM and DT in EM and PM South Indian subjects.

	EM	PM	P value
<i>Age(y)</i>			
Mean \pm SD	21.76 \pm 6.34	21.2 \pm 2.68	NS ^a
Range	16 -55	19 -25	
<i>BMI</i>			
Mean \pm SD	20.98 \pm 3.1	22.04 \pm 2.46	NS ^a
Range	12.74 - 38.22	19.61 -24.97	
<i>DM output (μmol/8h)</i>			
Mean \pm SD	1.29 \pm 1.7	05.06 \pm 06.49	0.0001 ^b
Range	0.23 - 3.48	0.56 - 19.44	
<i>Dose recovered as DM (%)</i>			
Mean \pm SD	1.51 \pm 1.99	05.94 \pm 07.62	0.0001 ^b
Range	0.28 - 4.31	0.66 - 22.84	
<i>DT output (μmol/8h)</i>			
Mean \pm SD	25.36 \pm 17.1	2.28 \pm 4.81	0.003 ^b
Range	0.47 - 71.9	0.06 -10.88	
<i>Dose recovered as DT (%)</i>			
Mean \pm SD	29.80 \pm 20.09	2.68 \pm 5.65	0.003 ^b
Range	0.58 - 84.49	0.07 - 12.78	
<i>MR</i>			
Mean \pm SD	0.054 \pm 0.04	1.997 \pm 2.25	0.003 ^b
Range	0.0024 - 0.246	0.325 - 7.95	

DM= dextromethorphan, DT= dextropran, MR= metabolic ratio,

PM= poor metaboliser, EM= extensive metabolisers, BMI= Body Mass Index

NS= Not significant, a = unpaired t-test and b = Mann-Whitney test

Table 9: Allele frequency according to Hardy Weinberg Law in the four South Indian states

State	Mutant allele %	Wild type %	Heterozygotes %
Kerala	21.9	78.0	34.2
Karnataka	20.0	80.0	32.0
Andhra Pradesh	13.4	86.6	23.2
Tamil Nadu	19.0	81.0	30.7
Mean	18.8	81.2	30.5

Table 10: Frequency of CYP2D6 PM reported in South Asia

Location of study (year)	Probe	n	PM(%)	95% CI	Reference No
Maharashtra (1984)	db	147	2	0.4-5	103
Sri Lanka (1994)	db	111	0	--	104
Chandigarh (1998)	dm	100	3	0.33-6.3	8
Andhra Pradesh (1999) (Telengana)	dm	146	3.2	0.95-7.5	9
Andhra Pradesh (2000) (Andhra)	dm	111	1.8	1.06-6.08	*
Kerala (2000)	dm	104	4.8	1.6-10.9	*
Karnataka (2000)	dm	100	4	1.1-9.9	*
Tamil Nadu (2000)	dm	139	3.6	1.2-8.2	*

n= number, PM= poor metaboliser, CI= confidence of interval

db= debrisoquin, dm= dextromethorphan, * = Present study

Figure11: Frequency distribution histogram of 454 South Indian subject:

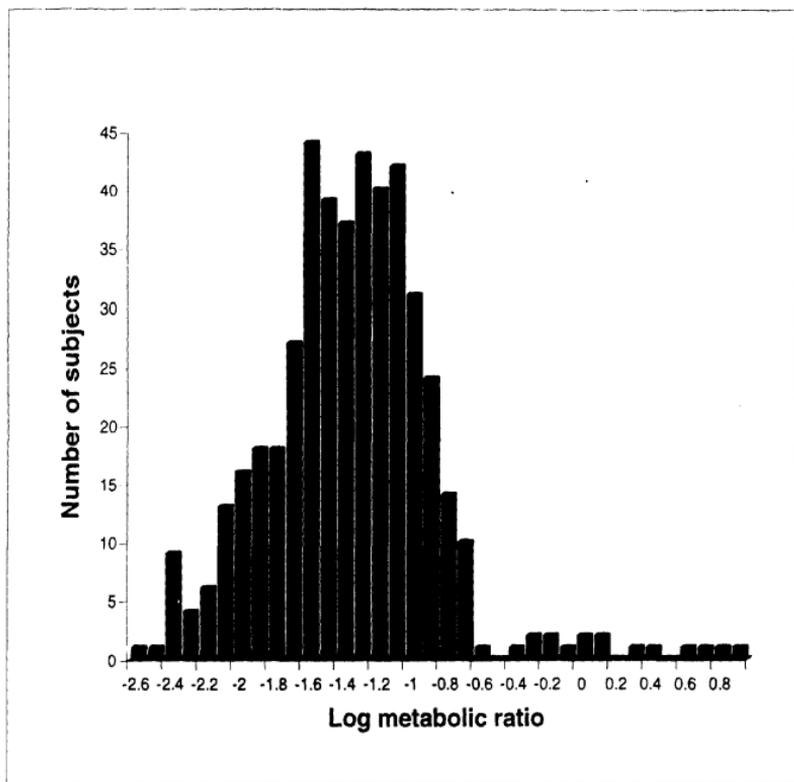


Figure 12: Probit plot of 454 South Indian subjects

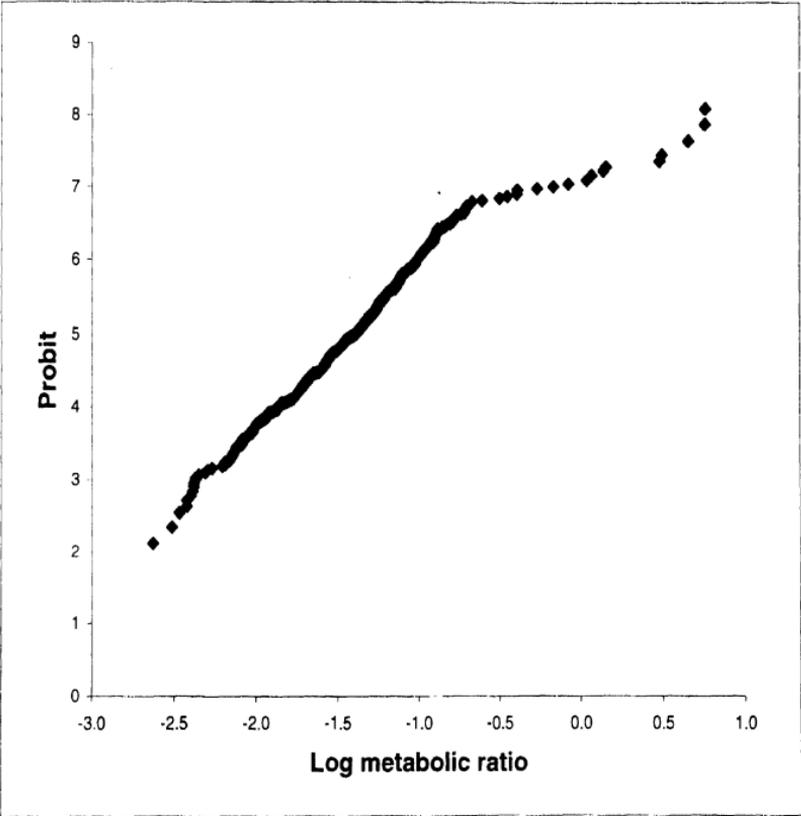


Figure 13: NTV Plot of 454 South Indian subjects

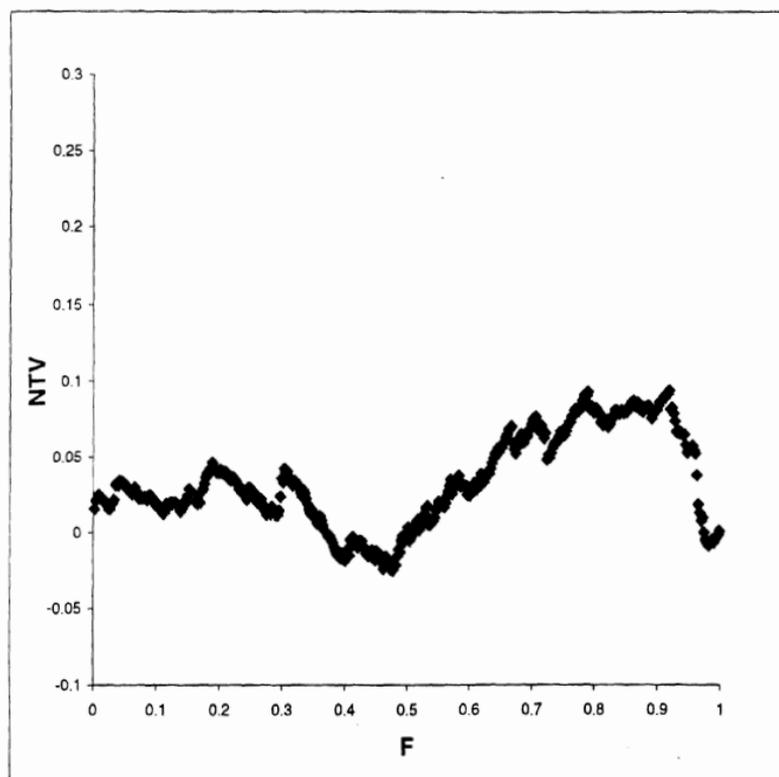


Figure 14: Frequency distribution histogram of 104 Kerala subjects

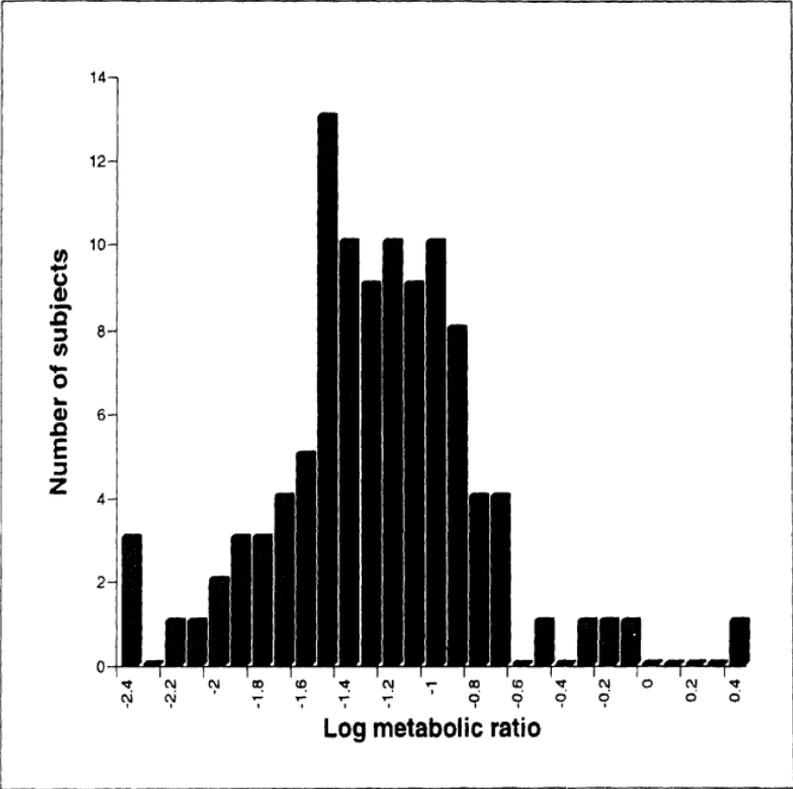


Figure 15: Probit plot of 104 Kerala subjects

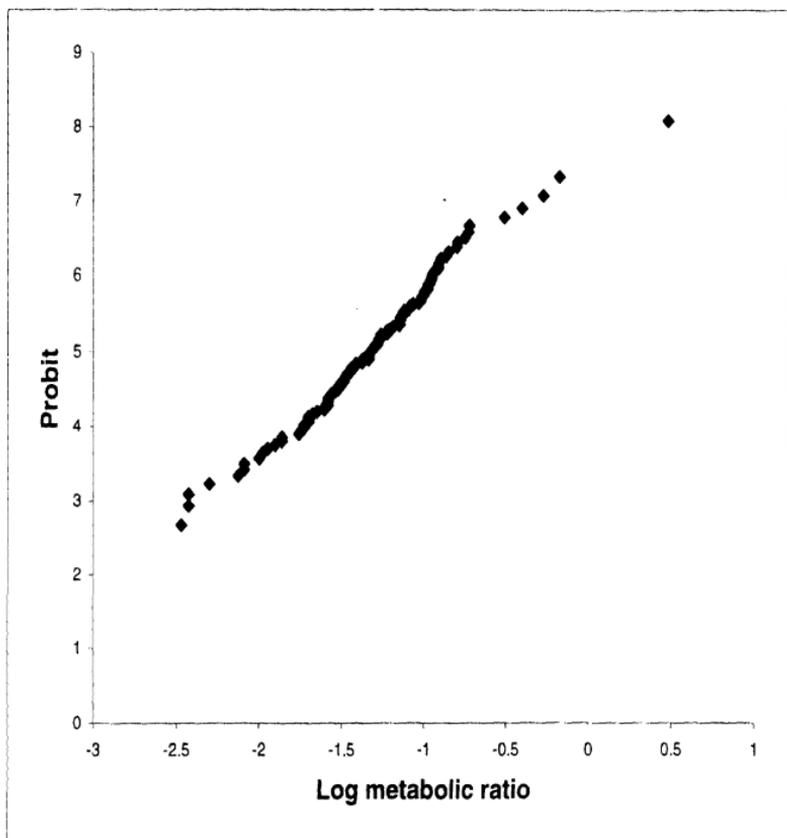


Figure 16: NTV plot of 104 Kerala subjects

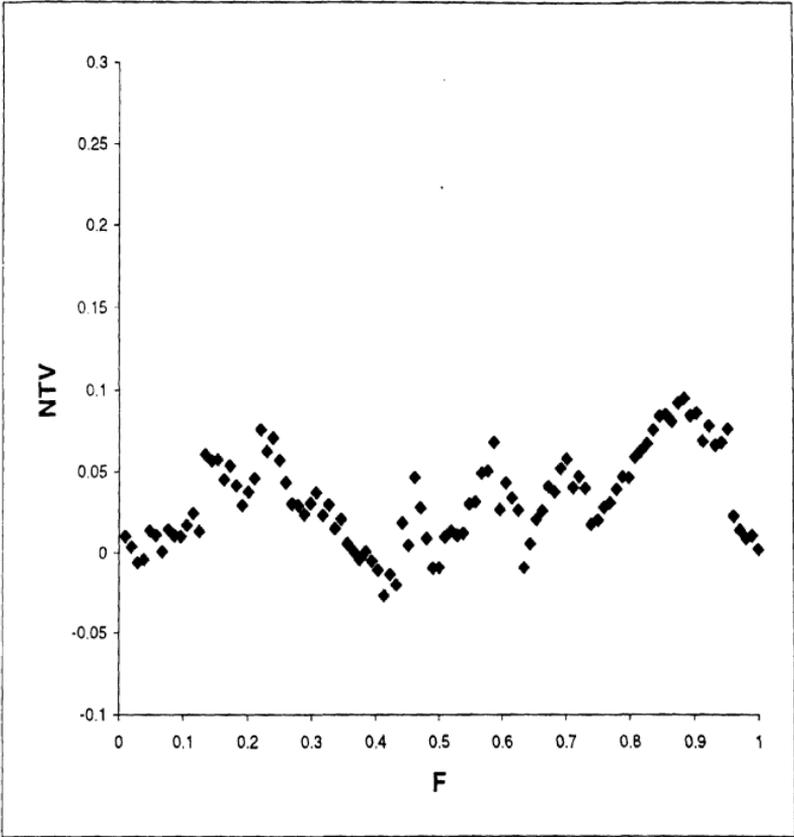


Figure 17: Frequency distribution histogram of 100 Karnataka subjects

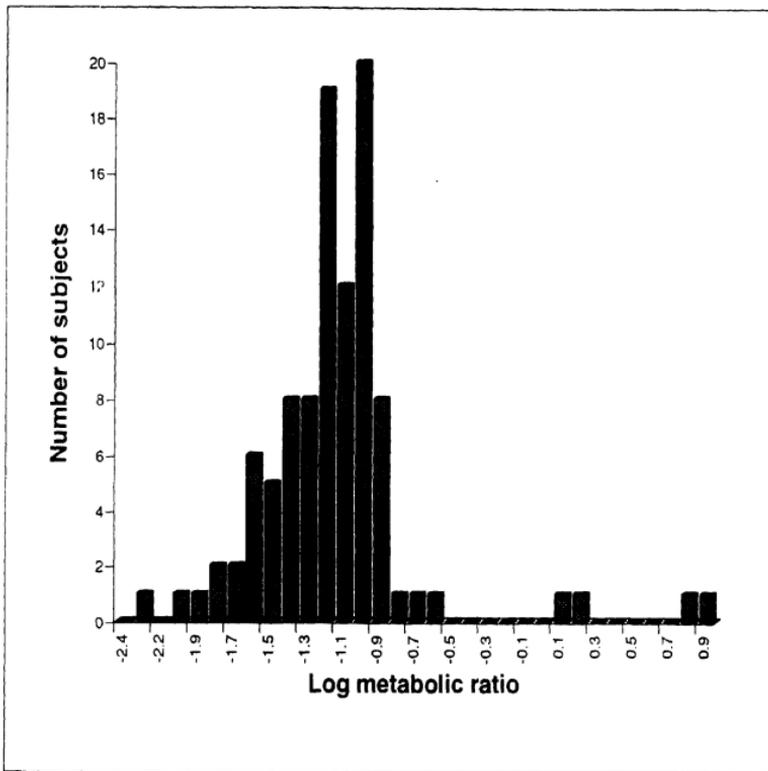


Figure 18: Probit plot of 100 Karnataka subjects

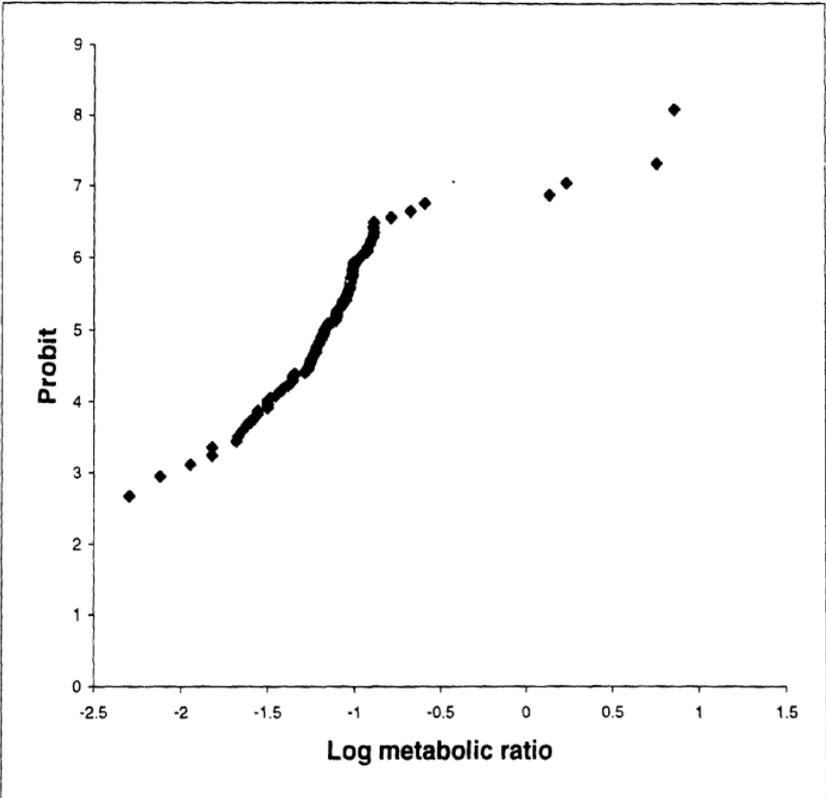


Figure 19: NTV plot of 100 Karnataka subjects

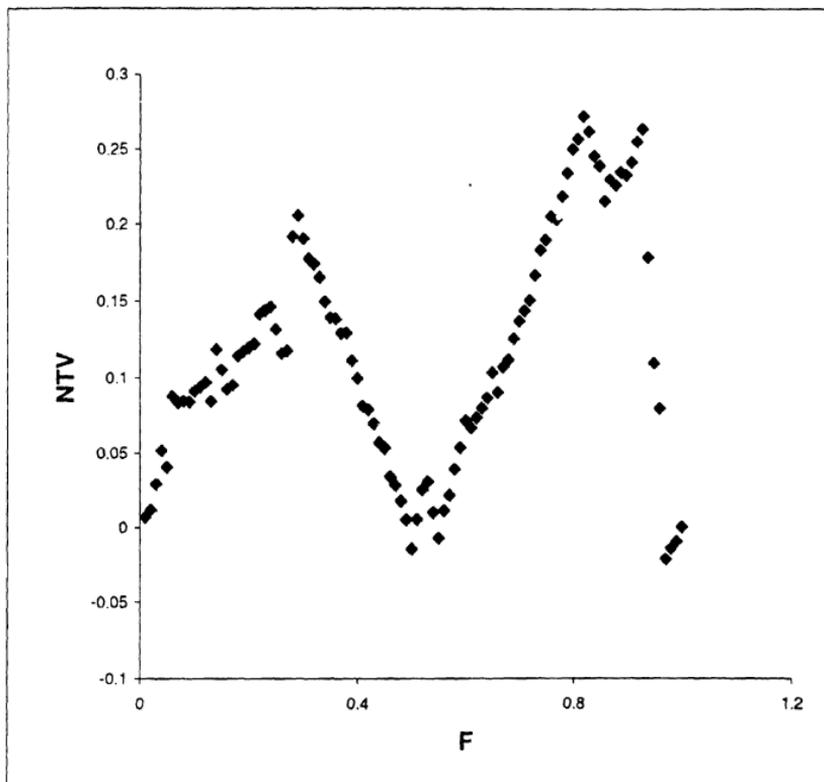


Figure 20: Frequency distribution histogram of 111 Andhra Pradesh subjects

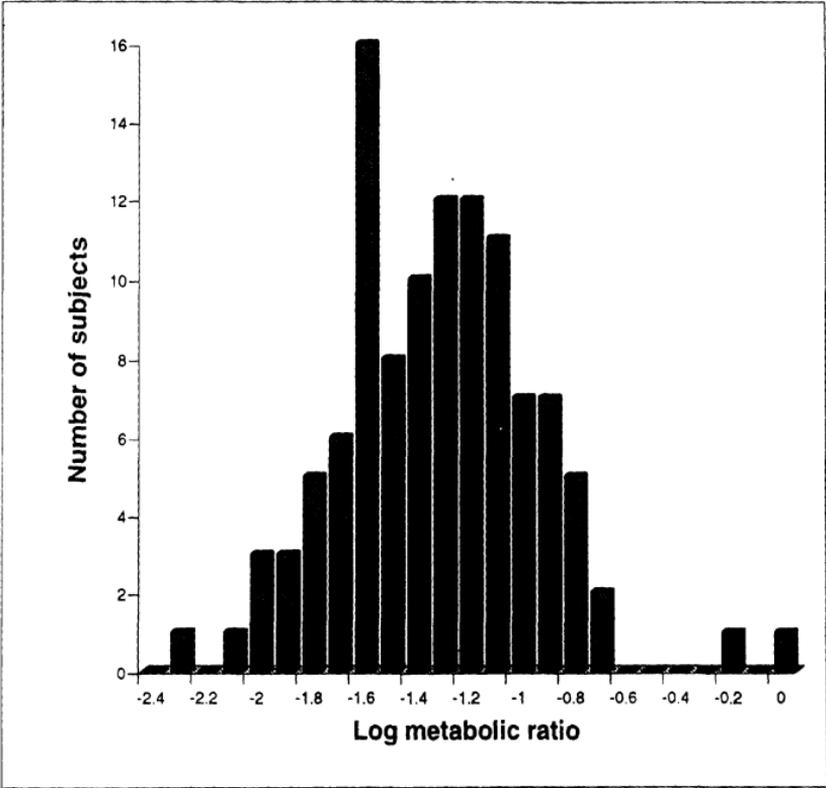


Figure 21: Probit plot of 111 Andhra Pradesh subjects

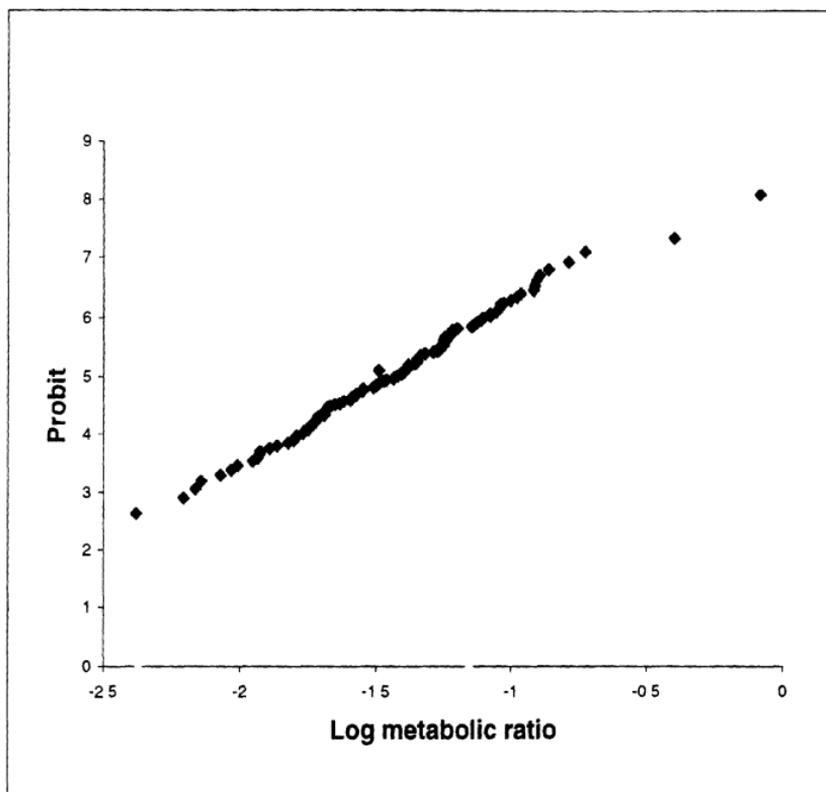


Figure 22: NTV plot of 111 Andhra Pradesh subjects

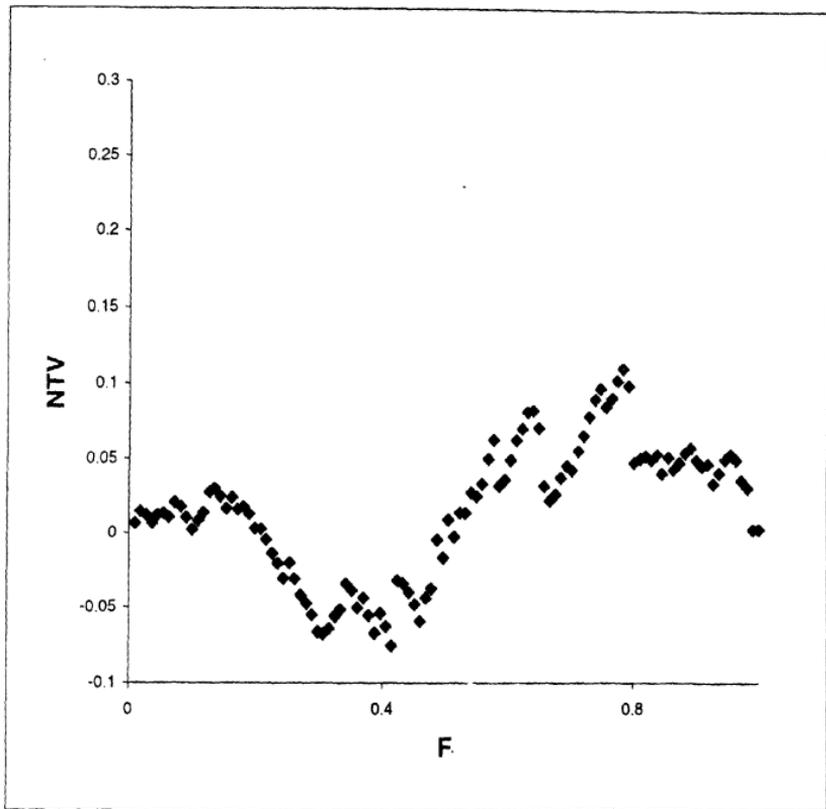


Figure 23: Frequency distribution histogram of 139 Tamil subjects

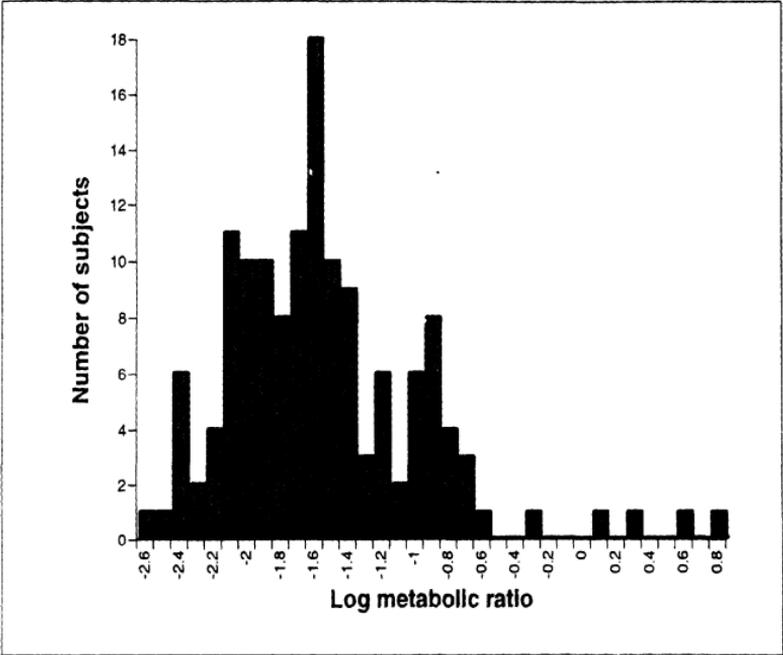


Figure 24: Probit plot of 139 Tamil subjects

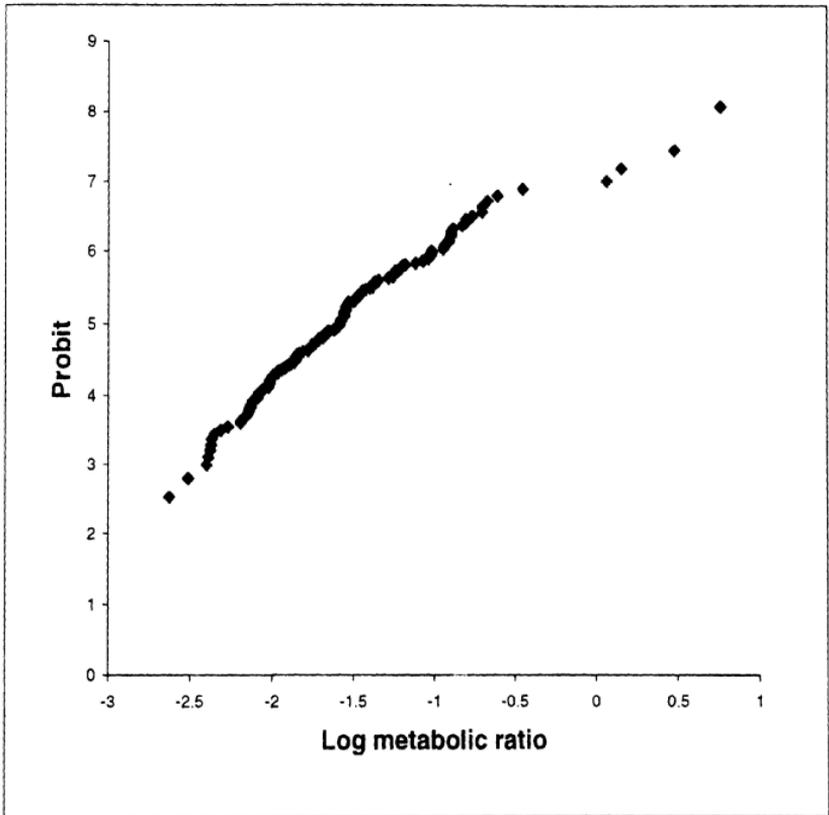


Figure 25: NTV plot of 139 Tamil subjects

