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Evaluation of CYP2D6 phenotype in the Yoruba Nigerian population

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Abstract

Background: There is a lack of information on CYP2D6, a major metabolizing enzyme, in Africa ethnic nationalities. The objective was to determine CYP2D6 phenotype in Yoruba Nigerians using dextromethorphan (DEX).

Method: A total of 89 healthy volunteers received 30 mg of DEX orally followed by blood and urine sample collection at 3-hour and over 8 hours post-dose, respectively. DEX and dextrorphan (DOR) concentrations were determined using High Performance Liquid Chromatography (HPLC). The metabolic ratio (MR, DEX/DOR) were plotted for the phenotype determination.

Results: The log MR that separated poor (PMs) from normal metabolizers (NMs) was 0.28 and 0.75 for urine and plasma, respectively. Two subjects (2.3%) identified as PMs had a mean MR of 17 and 3.2 in plasma and urine, significantly higher than that of NMs ($p<0.0001$). A positive correlation between urine and plasma MR was noted.

Conclusion: The prevalence of PMs in the Yoruba Nigerians was similar to that reported among blacks.

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Declaration of Interest

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Keywords

CYP2D6; dextromethorphan; metabolic ratio; phenotyping; Yoruba Nigerian

1 Introduction

CYP2D6 is responsible for the biotransformation of about 25% of clinically used drugs [1] including antidepressants (e.g. amitriptyline), antiarrhythmics (e.g. flecainide), antipsychotics (e.g. risperidone), β-adrenergic blockers (e.g. metoprolol) and miscellaneous drugs including codeine, tramadol, and tamoxifen[2]. CYP2D6 gene is highly polymorphic, and to date, more than 140 alleles have been discovered (www.cypalleles.ki.se/CYP2D6.htm). The phenotypic expression of these alleles have been grouped as: poor metabolizers (PMs), intermediate metabolizers (IMs), normal metabolizers (NMs), rapid metabolizers (RMs) and ultrarapid metabolizers (UMs) using such probe drugs as debrisoquine, dextromethorphan, sparteine, tramadol and metoprolol [3–5]. Of these probe drugs, dextromethorphan (DEX) appears favoured as it is relatively accessible and well tolerated when compared to the others [6–8]. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) is the most preferred for the analysis of dextromethorphan and its metabolites, but in its absence high performance liquid chromatography (HPLC) with florescence detector is better than ultraviolet (UV) detector.

CYP2D6 polymorphisms may result in up to a 30–40 fold difference in substrate clearance resulting in plasma concentrations that are outside the therapeutic range in patients [9, 10]. The clinical consequence of this may include concentration-related drug toxicity and or therapeutic failure [11, 12]. Therefore, adverse drug reactions resulting from poor drug handling due to enzyme polymorphisms should be preventable if appropriate dosing could be achieved. In this vein, therapeutic failure, and even economic-related losses may also be reduced. It is noteworthy that substantial drug-related morbidity and mortality are recorded yearly. For example, fatal adverse drug reactions were estimated as the 7th leading cause of death in Sweden in 2001 [13].

The prevalence of CYP2D6 phenotypes varies among different populations and races: studies showed 7– 10% PMs among Caucasians [14, 15], 1–2% PMs in the Asian populations [16, 17], 6.9% PMs in the Cuban populations [18], 3.1% PMs in the Ecuadorian populations[19], and 1–4% PMs population in Africa [20–23]. In Nigeria, few available studies among the many ethnic populations indicate a prevalence of PMs of 0–3.5% [24, 25].

The 2006 national census revealed that Nigeria has over 154 million people belonging to major and minor ethnic nationalities [26]. Of this, the Yoruba, a major ethnic nationality, had a population of over 32 million making up over one fifth of the Nigerian population. (www.mapsofworld.com/Nigerian) The Yoruba Nigerians are indigenous to the southwestern Nigeria. This present study, therefore, was aimed at evaluating single 3-hour plasma sample in the determination of CYP2D6 phenotype among the Yoruba Nigerians in order to identify poor metabolizers. Furthermore, the utility of urine samples for phenotype evaluation was explored by determining the metabolic ratio of dextromethorphan in urine compared to that calculated from the plasma samples.

2 Methods

2.1 Setting

Although Nigeria has over 350 ethnic nationalities, the three major ethnic groups; namely Hausa-Fulani, Yoruba, and Ibo constitute over 60% of the entire population. Yorubas are native to the Southwestern and some part of the Northcentral Nigeria, and they constitute about 21% of the total population of Nigeria. The study was conducted at the University College Hospital, Ibadan, Nigeria, a foremost tertiary health facility in the country. Ibadan, is the heartland and political headquarters of the Yoruba ethnic group in Nigeria.

2.2 Study Population and Subject Selection

Subjects' recruitment was through informal contacts and invitation through social functions and community meetings within and around Ibadan, Oyo State, Nigeria. The primary eligibility criterion was that the participant was, at least, a third generation Yoruba Nigerian with maternal and paternal parents and grandparents being of Yoruba extraction; in good health as confirmed by medical history, physical examination and laboratory investigations. The participants were unrelated. Other inclusion criteria were age between 18 and 50 years, informed consent of the participants, non-smoking, not on any medication including herbal preparations. Exclusion criteria were the chronic use of alcohol or any other recreational drugs, use of oral contraceptives, pregnancy, lactation, known/history suggestive of allergy to dextromethorphan and related drugs.

Eligible participants were interviewed on their medical history; physically examined (including blood pressure, height and weight measurement); blood (5mL) and urine samples were collected for laboratory investigations such as complete blood count, liver and renal function tests, and urinalysis, to further ascertain fitness for the study. The participants were enrolled in batches of 10 or 12 on each of the recruiting days, between 2 September and 30 September 2014 taking full cognizance of their convenience.

The study received ethical approval from the Joint University of Ibadan/University College Hospital (UI/UCH) Ethical Review Committee (UI/EC/12/0186), and was conducted in accordance with the principle of Helsinki declaration. Each participant, prior to the commencement of the study and following a detailed explanation of nature signed an informed consent. Due to the relatively small sample size, the primary objective was modified to identify poor metabolizers.

2.3 Drug administration and sample collection

Following an overnight fast, participants were requested to completely empty their bladder after which 30 mg dextromethorphan hydrobromide, in syrup form, was given orally. Three hours post dextromethorphan administration, 5 mL of blood was collected, immediately centrifuged and the plasma transferred to separate plain plasma tubes. Also urine samples were collected over the first 8-hour period into a container having 2 g of ascorbic acid for acidification. The total urine voided by each participant was recorded before storing 15 mL aliquots in a container with 20 mg ascorbic acid. The plasma and urine samples which were initially stored at −20°C and was later transferred to a −80°C ultralow freezer until analysis.

During the nine months' period of storage, daily charting of the temperature of the freezer was done morning and evening. In addition to the generator, an inverter was in place as an immediate backup in the case of power failure. The samples were shipped in liquid nitrogen from the University College Hospital, Ibadan to the Central Science Laboratory (CSL) at the Obafemi Awolowo University (OAU), Ile-Ife, Nigeria where the analyses were done.

2.4 Drug Analysis

Dextromethorphan and dextrorphan concentrations in plasma and urine were analysed using a previously reported methods by Zimova *et al* [27] and Jurica *et al* [28] with modifications. Dextromethorphan hydrobromide syrup (Tussipect^{R}), (Belgium) 1.5mg/mL was supplied, in part, free by Benjamin Michael Pharmacy, Lagos, Nigeria. The following reference standards, all of HPLC grade, were used including dextromethorphan (drug), dextrorphan (metabolite) and levallorphan (internal standard), β-glucuronidase (Helix Pomata), acetonitrile and methanol, all from the Sigma Aldrich, Steinheim, Germany. Analytical grade of anhydrous sodium carbonate, anhydrous sodium acetate, orthophosphoric acid, potassium dihydrogen orthophosphate (KH_2P0_4), hydrochloric acid and ascorbic acid, all from the British Drug Houses (BDH) Chemical Limited, England. Other chemicals used included the analytical grade of hexane and n-butanol (Scharlau SL Gato Pèrez, Barcelona, Spain) and triethylamine (Burgoyne Burbibges and Co, Mumbai, India). Briefly, the method involved addition of 0.4 mL sodium acetate (0.2 mol per liter) and 4000 units/sample β-glucuronidase to 1 mL of urine and incubated in water bath at 37 °C for 18 hours to enzymatically hydrolyse the sample. The mixture (1 mL) was pipetted into 10-mL screw-capped, tapered glass tubes followed by addition of 20μg/ml of internal standard and 4 mL Na_2CO_3 (0.5 mol/litre). Samples were extracted with 4 mL of hexane: n-butanol, 9:l(v/v) mixture. After vortexing and centrifuging, the organic layer was separated and kept, but the aqueous phase was reextracted. Both organic phases were subjected to re-extraction into 0.3 mL of 0.1M hydrochloric acid (HCL) (0.1M), and 50 μL of the aqueous phase was injected into the HPLC. Plasma and urine samples were subjected to the same protocol. The high performance liquid chromatographic system used consisted of an Agilent 1260 series HPLC system (Agilent Technologies, Germany) fitted with 1260 quaternary pump VL (model G1311C), an online vacuum degasser (Agilent, Japan), a manual injector valve ((Rheodyne, USA) with a 20 μl sample loop, and sample detector with a variable wavelength (VWD) (190–600nm) standard version (model G1311B). Data acquisition was enabled by an LC3D Chemstation software and window 2007 for system control. Chromatographic separation was achieved with an Eclipse plus C-18 reverse phase HPLC column, (3.5 μm particle size and 100 × 4.6 mm, i.d.), Agilent United States of America (USA). The mobile phase consisted of 30% Acetonitrile: 20% Methanol: 0.06% Triethylamine: 49.94% KH_2PO_4 (0.01 mol/litre), adjusted to pH of 3.2 by orthophosphoric acid (0.1 mol/litre). The flow rate was 1.5ml/min at the ambient temperature. The wavelength of the Ultraviolet-Visible Spectrophotometry (UV-VIS) detector used was 230nm.

Dextrorphan, levallorphan, and dextromethorphan were distinctly resolved eluting with retention time of 1.3, 1.7 and 3.5 minutes, respectively. The Intra-assay and Inter-assay coefficients of variation for both dextromethorphan and dextrorphan were less than 6% at concentrations of 0.5 and 2μg/mL (n=6). The limit of detection was 0.1 μg/mL while the

limits of quantitation was 0.2 μg/mL for both dextromethorphan and dextrorphan in the urine and plasma.

2.5 Data Analysis

The statistical analysis was performed using International Business Machines Corporation-Statistical Package for the Social Sciences (IBM SPSS) Statistics for Windows, version 22 and Microsoft Excel 2013. The socio-demographic variables were summarized using descriptive statistics (frequency and percentage). The age, height, weight and Body Mass Index (BMI) were computed with mean ± standard deviation. The molar concentrations of dextromethorphan and dextrorphan in the plasma and urine samples were measured and summarized as mean, and standard deviation. The metabolic ratio of dextromethorphan/dextrorphan ($MR_{DEX/DOR}$) was calculated by dividing the molar concentration of dextromethorphan (μg/mL) by the molar concentration of dextrorphan (μg/mL). The log $MR_{DEX/DOR}$ at 3-hour for plasma and 8-hour for urine were calculated and used as the index of CYP2D6 activity. A probit plot of log MR was constructed for plasma and urine, and the anti-mode (cut-off points) that separated the phenotypes (poor and normal metabolizers) were obtained from the graph. The probit plot was constructed with Microsoft Excel 2013, with log MR on the X-axis and probability % on Y-axis on the semi-logarithm graph. The probability % was calculated for each sample (plasma and urine) using the equation: Probability %, P = 100(i-0.5)/T [29], where i = rank of the participant log MR (from 1–89) and T = total number of participants (i.e. 89). Trend lines were added to the probit plot to get the best line of fit. Based on the best line of fit, a polynomial equation of regression was obtained, and intercept at X-axis was considered as the log MR separating poor metabolizers (PMs) from normal metabolizers (NMs). Pearson's correlation was used to compare the quantitative variables (plasma and urinary metabolic ratios, age, body mass index) while the independent t-test was used to compare mean values in two groups such as male and female. The p-value was set at <0.05 for statistical significance.

3 Results

One hundred and four (110) consented to participate in the study but 11 individuals could not be enrolled for (apparent) inadequate renal function and/or ingestion of medicines or herbal remedies. Of the 99 participants, four samples could either not be found or urine collection discovered to be incomplete, and six samples were removed because the concentrations obtained for dextromethorphan and or dextrorphan were lower than than the limit of quantification (0.2 μg/mL). Of the 89 participants who completed the study per protocol with all relevant samples being available, 58(65.2%) were males, and 31(34.8%) were females. The mean age of the participants was 36.1±9.5 years. The mean weight, height and Body Mass Index (BMI) were 64±13.4 kg, 1.69±0.07 m and 22.4±4.2 kg/m^2, respectively. One participant reported mild drowsiness one-hour post-dose which resolved spontaneously without any intervention.

The mean concentrations of the dextromethorphan and dextrorphan in the 8-hour urine were 0.75±0.54μg/ml and 1.01±0.51 μg/mL. respectively. The plasma and urine concentrations of dextromethorphan and dextrorphan are summarized in **Table 1**.

The median (range) metabolic ratio and log MR in the 8-hour urine were 0.74 (0.001–4.2) and −0.13 (−2.9–0.6), respectively. There was no statistically significant gender difference in the mean MR urine (t=1.8, p=0.072). **Figure 1** provides a histogram of the frequency distribution of the metabolic ratio in the 8-hour urine. As shown in **Figure 2**, the cut-off (log MR) and the antimode for 8-hour urine separating the poor metabolizers (PMs) from extensive metabolizers(EMs) were 0.28 and 1.91 respectively. Two (2.3%) participants were classified as poor metabolizers (PMs).

The mean concentration of dextromethorphan in the 3-hour plasma was 3.4±1.8 μg/mL while that of the dextrorphan was 1.33±0.79 μg/mL. The median (range) of the metabolic ratio in the 3-hour plasma was 2.36 (1.4–26.5) while the median log MR was 0.37(0.1–1.4). The MR plasma was not significantly different between genders (p=0.072). **Figure 3** provides a histogram of the frequency distribution of the log MR of the 3-hour plasma samples. As shown in probit plot in **Figure 4**, the log MR that separated the normal metabolizers (NMs) from poor metabolizers (PMs) was 0.75, and the anti-mode was 5.6. Two (2.3%) participants were classified as poor metabolizers (PMs).

Both the (8-hour) urine and the plasma sample obtained 3 hours post-dose identified the two participants (2.3%) as poor metabolizer (PM) phenotype. The two PMs identified were male aged 25 and 27years, respectively. The plasma MRs of the PMs were 7.5 and 26.5, while their urine MRs were 2.2 and 4.2. The NMs has the median (range) plasma and urine MRs of 2.4(1.4–4.9) and 0.7 (0.01–1.8) respectively. There was a strong positive correlation between 8-hour urinary and 3-hour plasma metabolic ratios [r=0.77, p<0.0001, CI (0.22, 0.89)] but no significant correlation between age, body mass index and metabolic ratios as shown in **Table 2. Figure 5** shows the scatter plot displaying the linear relationship between the 3- hour plasma and 8-hour urine metabolic ratios.

4 Discussion

A single 3-hour plasma sample was employed in the determination of CYP2D6 among 89 unrelated healthy volunteers of Yoruba ethnic origin in Nigeria. The few existing studies on CYP2D6 phenotyping of specific ethnic nationalities in Nigeria used urine matrix hence the uniqueness of this study [24]. Interestingly, the two (2.3%) PMs identified using 3-hour plasma sample were confirmed by the urine sample, and similar to the documented prevalence of PMs in our area of study. The specific objectives of the study did not include determination of other possible phenotypes such as ultra-rapid and intermediate metabolizers though, it would appear from the various plots that the NMs could be further separated into two sub-groups.

The Yoruba Nigerians are considered a homogenous population and of common ancestry. Considerable efforts were made to ensure that only participants with sufficiently long Yoruba ancestry were enrolled. In addition, inclusion criteria also took into cognizance other factors that are known to influence the rate and extent of drug metabolism, and therefore potential confounders. For example, within the age range of the participants, there was a lack of correlation between age and the metabolic ratios of the participants. It is also noteworthy that the two poor metabolizers that were identified were aged 25 and 27 years.

The age range in this study was similar to previous studies investigating CYP2D6 phenotype [24, 30, 31]. The established tolerability profile of dextromethorphan was further demonstrated by the fact that only one (~1%) participant in our study had mild and transient drowsiness [24, 28, 31–33]. In CYP2D6 phenotyping, the requisite cutoff(s) may be obtained by subjecting data to a probit analysis, Receiver Operating Characteristics (ROC) or other similar statistical analysis [28, 33, 34]. On the other hand, some studies have simply adopted cut-off [35, 36]. The former option was chosen in this case as there is no immediate record of an identical study, that is, determination of CYP2D6 phenotype in the Yoruba Nigerians using a single plasma sample. Further, some researchers have suggested against the utilization of a "universal" cutoff as it may be misleading [28, 37]. The histogram reveals the bimodal distribution and the same was reinforced by the probit plots similar to the previous findings [33, 37]. Studies have reported different MR cutoffs separating the PMs from NMs, while some adopted a universal MR of 0.3 obtained among Caucasians in both 8-hour urine [32, 35], and 3-hour plasma[38, 39]. Some studies reported MR cutoffs for 8-hour urine were 0.3 in Swiss[40], Cuba[18], and Gabon[41], 0.24 in Spain (Spaniards) [42], and 0.32 by Ebeshi et al. in Nigeria [24]. For the 3-hour plasma, some reported log MR cutoff was 0.742 by Jurica et al. in Czech Republic [28], 0.8 by Dorado et al. in Ecuador[43] and 0.142 by Gogtay et al. reported in India [33].

This study explored the comparability of the well-established non-invasive but time-consuming urine versus the relatively invasive but less cumbersome single plasma collection methods. Evaluation of drug metabolism phenotypes has evolved. Though hitherto, a 24-hour urine collection was required, recent studies have used 4 and 8-hour urine sample collection [44, 45]. In addition, a single 3-hour plasma sample has been found to be an equally useful alternative [28, 46]. For emphasis, previous studies have found plasma matrix for phenotyping with dextromethorphan accurate, convenient and more rapid than the standard urine approach [28, 46–48]. Besides, the use of single plasma sample appears to assist in avoiding potential error that preferential accumulation of metabolites due to impaired renal function may introduce in the determination of metabolic ratio.

The frequency of CYP2D6 poor metabolizers obtained in this study is similar to previous studies in Nigeria and other African countries. Ebeshi *et al.* obtained a prevalence of 3.5% poor metabolizers with urinary dextromethorphan/dextrorphan metabolic ratio among heterogeneous Nigerians[24]. Other studies in Africa also showed a poor metabolizer prevalence of 1.2% in South Africa [31] and 1–4% in other African countries [20, 21, 49]. These findings are similar to that of Asian countries with a poor metabolizer prevalence of 1–4% [16, 17, 33]. The prevalence of poor metabolizer phenotype among the African-Americans appears to be slightly higher at between 5.3% and 7.7%, [50, 51] and even higher still among the Caucasians, 7–10% [28, 36]. The clinical implications of poor metabolizers include slower drug metabolism with potential for adverse drug interactions. There may also be the slower conversion of a prodrug to active metabolites with potential lower efficacy [52, 53].

There were some challenges with the assay of DEX and DOR, particularly for resource-poor countries where sophisticated analytic facilities may be lacking. Although, HPLC with UV detection used in this study was not best suited for determination of dextromethorphan and

dextrorphan when compared to LC-MS/MS or HPLC florescent detector with superior sensitivity, this method described by Zimova and Jurica with modifications appeared to be adequate particularly with the assumption that the two major metabolites would have been affected to similar extent.

An accurate determination of CYP2D6-genotype predicted phenotype is required in the clinical settings for precision medicine. However, CYP2D6 genotype-phenotype matching alone may not produce a desirable result, as studies have reported discordance in genotype-predicted PMs [54–56], and recent studies also suggested that CYP2D6 genotype was not a good predictor of ultra rapid metabolism[18, 19, 57]. Gaedigk et al.[58], and Zineh et al. [59], have proposed an Activity Score (AS) that are calculated from the CYP2D6 gene and use to determine the CYP2D6 metabolic activities of an individual. Vanwong et al. using risperidone as a probe [60], Puangpetch et al. [61], in Thailand, and Dodgen et al. in South Africa [31], reported a good correlation between AS, CYP2D6 genotypes and measured phenotypes.

We recognized that a genotype-phenotype pairing and prediction of CYP2D6 enzyme activities with AS would have enhanced a study like this but the genotype study could not be performed due to resource constraint. Although limited by the small sample size and rather conformational findings, the relevance of the present study could be justified by lmited information of CYP2D6 phenotypes available from only two completed studies in Yoruba Nigerians who constitute 40 million people accounting for ~20% Nigerian population. Additionally, dextromethorphan as a common probe drug for CYP2D6 phenotyping might not be optimal for estimating *in vivo* enzyme activity due to its limitation such as no linear correlation existing between enzyme activity and urinary ratios and its dependence on renal and hepatic transport. Moreover, CYP3A4/5 polymorphisms also contribute to the variability in the clearance of dextromethorphan. Nevertheless, the use of dextromethorphan in this study was largely owing to its accessibility in lower and mid-income settings. Despite other limitations including the relatively poor distinction between NM and PM or ultra rapid from EM phenotypes based on the urinary metabolic ratio, we were able to identify PM using this approach. It is also noted that this was the very first report where DEX in plasma was used to phenotype CYP2D6 in Nigerian populations. This study revealed a significant positive correlation between 8-hour urine and 3-hour plasma metabolic ratios and indeed demonstrated that a single 3-hour plasma sample following administration of DEX to Yoruba Nigerians appears adequate in segregating NMs and PMs on the CYP2D6 metabolized drugs.

5 Conclusions

Single 3-hour plasma sample following administration of DEX revealed similar PMs to 8-hour urine sample among the 89 Yoruba ethnic group of Nigerians studied.

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6.

Key issues

- CY2D6 is highly polymorphic and responsible for the metabolism of about 25% of all clinically used drugs.
- Dextromethorphan has been widely utilized as a probe for CYP2D6 phenotype.
- There is a dearth of information on CYP2D6 phenotype regarding Africa ethnic nationalities including the Yoruba Nigerian.
- The metabolic ratios of dextromethorphan in urine and plasma could be used to separate poor CYP2D6 metabolizers from extensive metabolizers.
- A significant positive correlation between urine and plasma metabolic ratios was noted.
- Approximately 2–3% of the Yoruba Nigerians identified as poor metabolizers can be expected to benefit from CYP2D6 phenotype evaluation to avoid drug-related adverse effects.

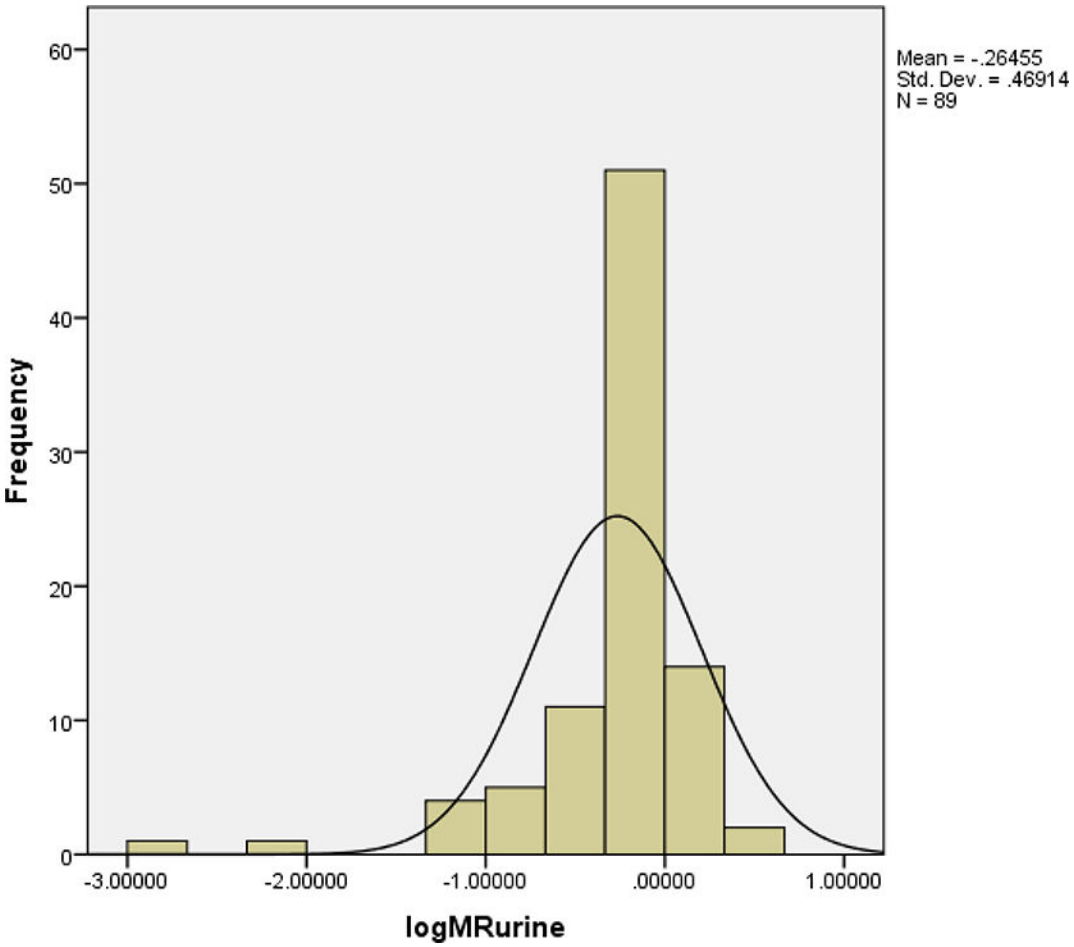

Figure 1.
Histogram of the frequency distribution of the log MR in the 8-hour urine.

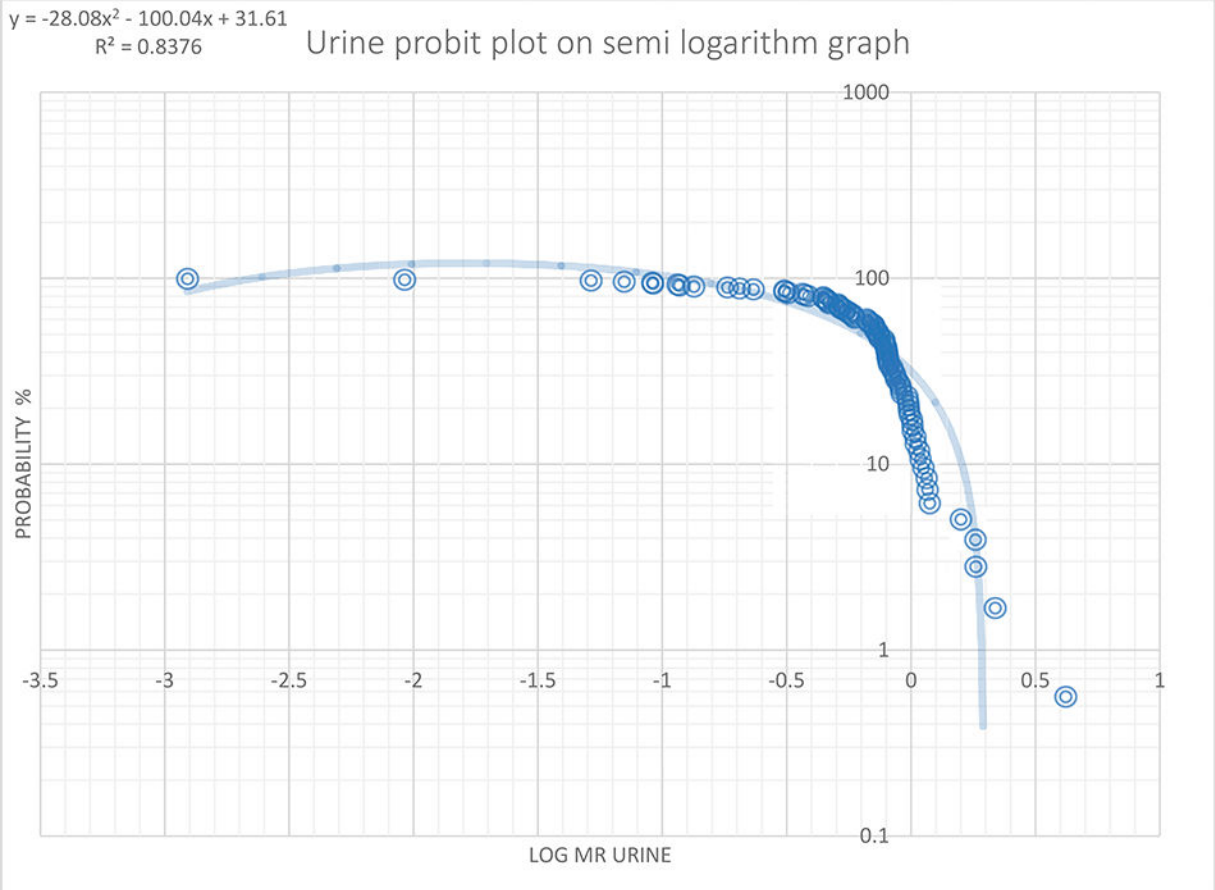

Figure 2:

Probit plot of metabolic ratio (n=89) in 8-hour urine samples

The probit plotted using the semi-log graph of probability percent (probability %) against the log MR and the trend line best fit plotted. The log MR (0.28) when the trend line crosses the x-axis was chosen as the anti-mode (1.91) by converting back to MR (i.e. the antilog of log MR) separating the poor metabolizers from the normal metabolizers.

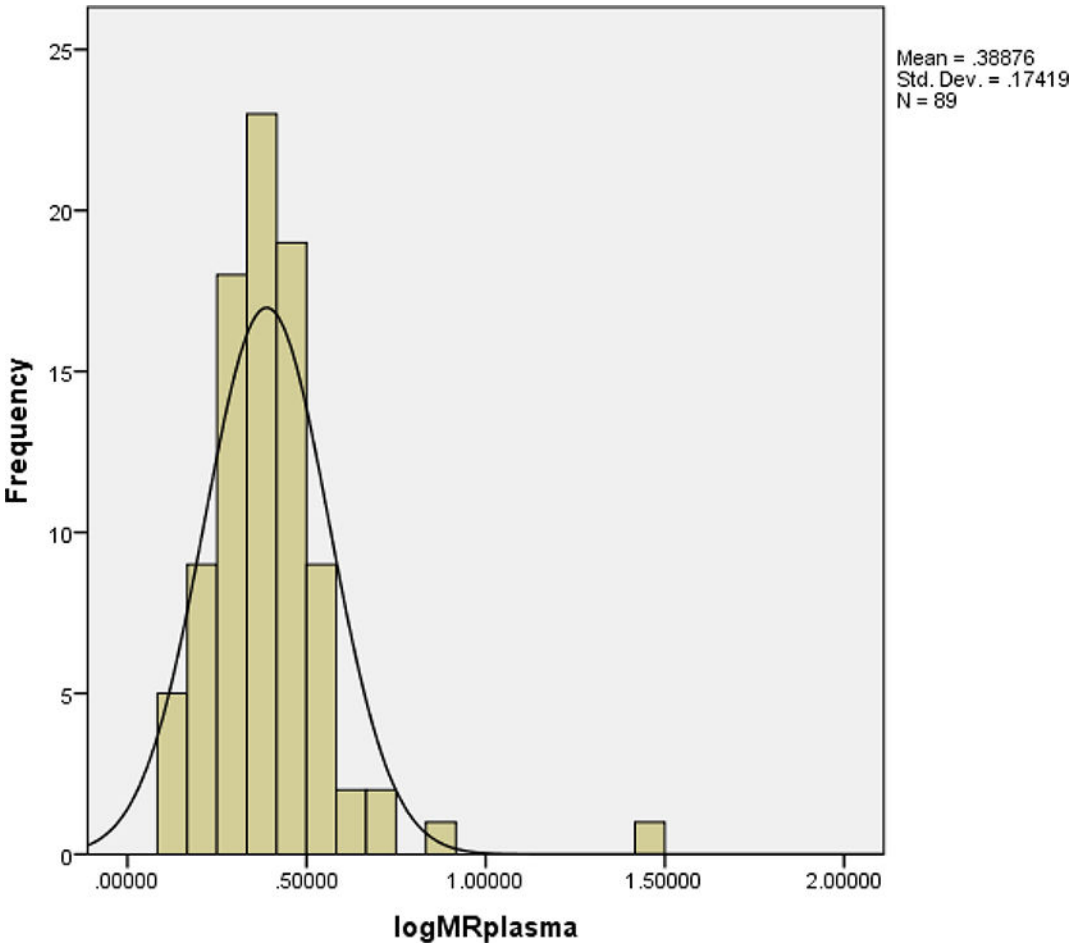

Figure 3.
Histogram of the frequency distribution of the log MR in 3-hour plasma.

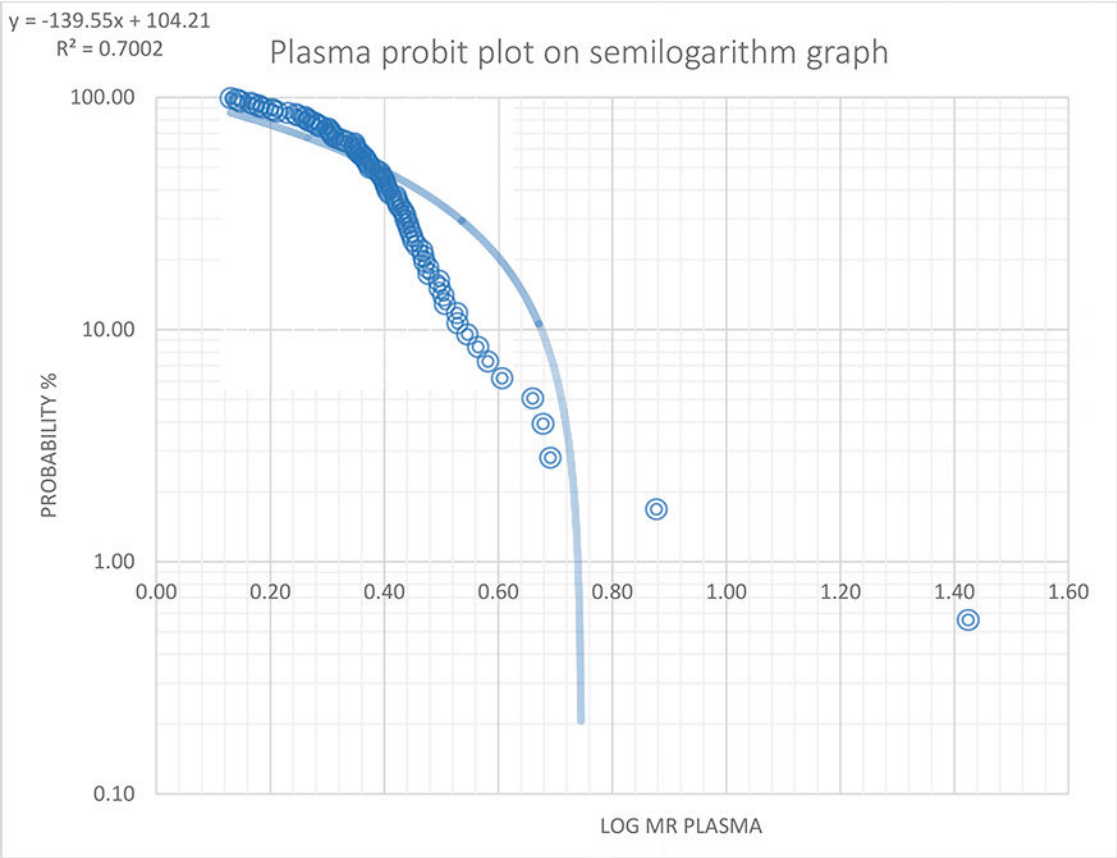

Figure 4.

Probit plot of metabolic ratio (n=89) in 3-hour plasma samples

The probit plotted using the semi-log graph of probability percent (probability %) against the plasma log MR and the trend line best fit plotted. The log MR (0.75) corresponding to MR of 5.6 when the trend line crosses the x-axis was chosen as the cut-off separating the poor metabolizers from the normal metabolizers.

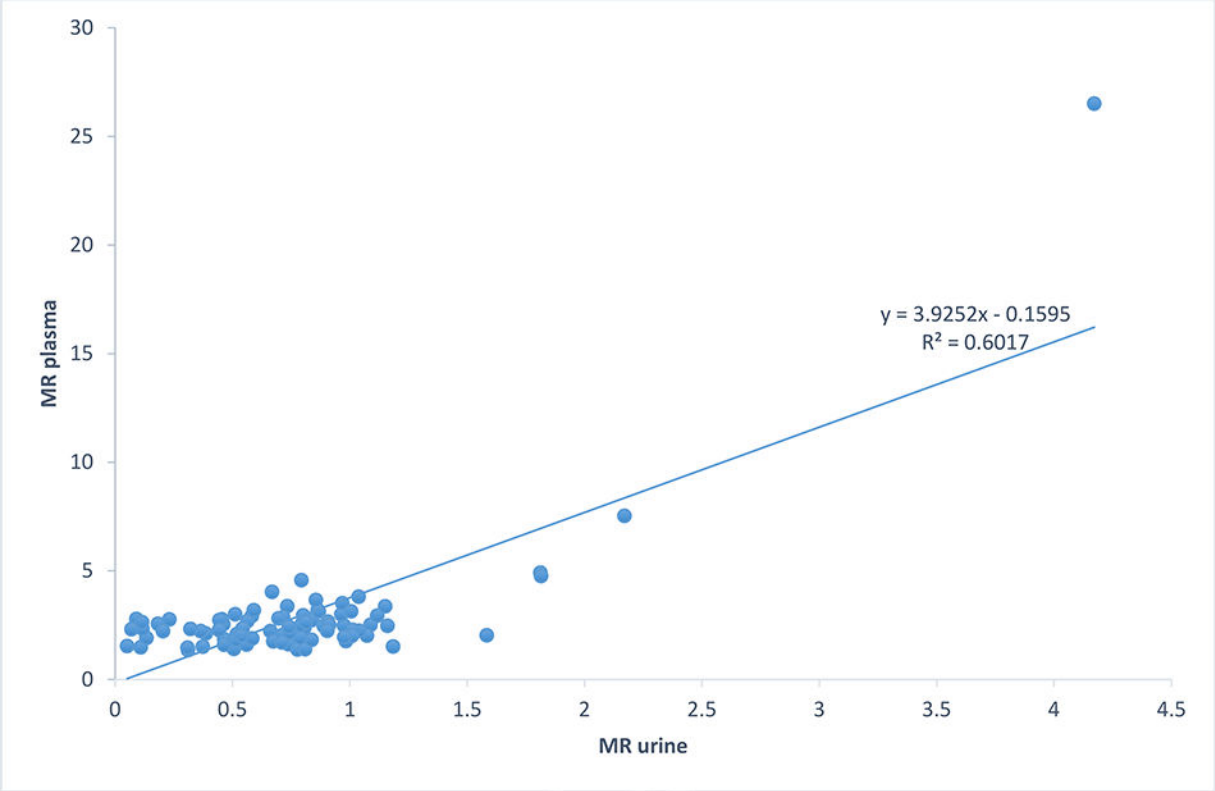

Figure 5:
Scatter plot showing the correlation between the Metabolic Ratios(MR) of the 3-hour plasma and 8-hour urine

Table 1:

Plasma and urine concentrations of dextromethorphan and dextrorphan in the participants

Variable(ug/ml)	Mean±SD	Median(range)
8-hour urine dextromethorphan	0.75±0.54	0.74(0.2–2.56)
8- hour urine dextrorphan	1.01±0.51	0.95(0.36–3.90)
3-hour plasma dextromethorphan	3.14±1.78	2.5(0.51–9.00)
3-hour plasma Dextrorphan	1.33±0.79	1.03(0.2–4.22)

Table 2:

Correlation of the 8-hour urine MR, 3-hour plasma MRs, age and body mass index

Variable	Age(years)	BMI(kg/m2)	Urine MR	Plasma MR
Urine MR r(p-value)	0.14(0.188)	−0.09(0.43)	1	0.77(<0.0001)*
Plasma MR r(p-value)	0.09(0.359)	−0.04(0.622)	0.77(<0.0001)*	1

*Statistical significant