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Genetic Polymorphism of Cytochrome p450 (2C19) Enzyme in Iranian Baluch Ethnic Group.

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ABSTRACT

Interindividual differences in the metabolism of many drugs in humans are depended on genetic polymorphisms. The aim of this study was to determine the CYP2C19 genotype profiles of Baluch ethnic group by studying allelic differences and compare their frequencies with results of other studies in different populations. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism technique was used for Genotyping of CYP2C19 gene. One hundred and forty unrelated healthy Baluch origin people who were referred to Health Center in Chahbahar, in Sistan and Baluchestan province enrolled in the study. The allele frequency of CYP2C19*1, CYP2C19*2 and CYP2C19*3 were 88.93%, 10% and 1.07%, respectively. 78.57% of subjects were with CYP2C19*1/*1 genotype. 20%, 0.71% and 0.71% subjects were with CYP2C19*1/*2, CYP2C19*1/*3 and CYP2C19*3/*3 genotypes, respectively. 0.71%, 20.71% and 78.57% of subjects were poor-, Intermediate- and extensive metabolisers. The allelic variants of CYP2C19*2 and CYP2C19*3 in Baluch ethnic group are comparable to other different population. CYP2C19*1 was the most frequently allele (88.93%) in our subjects. It is important to study the clinical significance of those variations for optimal drugs dosage which metabolize by the CYP2C19 enzymes in response to different substrates such as S-mephenytoin, methylphenobarbital, omeprazole, phenytoin, imipramine, proguanil, propranolol, and diazepam.

Key Words: CYP2C19 genetic polymorphism, Baluch ethnic group, polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).

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INTRODUCTION

Interindividual differences in the metabolism of many drugs in humans are depended on genetic polymorphisms [1, 2]. It is important to define metabolic capacity in different ethnic groups by use of phenotyping or genotyping tests for a safer drug management. The cytochrome P450 enzymes (CYP) 2C9, 2C19 and 2D6 are the most drug metabolizing enzymes (via drug oxidation) [3, 4].There are at least four isoforms of 2C8, 2C9, 2C18 and 2C19. Their genes are located on chromosome 10. From human CYP2C isoforms, CYP2C19 is the most abundant which metabolizes many important drugs, such as *S*-warfarin, phenytoin and losartan [5]. Studies have indicated that enzymatic activity changes with alterations in the amino acid sequence [6]. Different allelic variants (*CYP2C19*1*, *CYP2C19*2*, *CYP2C19*3*) of enzyme with different catalytic activity have been shown [5]. The definition of allele distribution of *CYP2C19* genetic polymorphism may allow using optimal drug dosage in pharmacological treatments. The most important alleles of CYP2C19 isoenzymes are CYP2C19*2/*3 and CYP2C19*2/*3 allelic variants. The wild-type alleles are indicated as 'CYP2C19*1'. Allelic variants of CYP2C19*2/*3 and CYP2C19*2/*3 show almost10–40% and 5–15% of the activity of CYP2C19*1, respectively [7]. The frequencies of the CYP2C19*2 and CYP2C19*3 alleles change from 8 to12% and from 3 to 8% among Caucasians, respectively [8, 9], which is lower in comparison with Orientals and Black Africans.[8, 10]. Studies have shown that the CYP2C9*2 allele has not been indicated in Han Chinese, Japanese and Taiwanese populations. The frequency of the CYP2C19*3 allele in Japanese (2.1%) and Taiwanese (1.7%) populations has been shown [11]. The frequency changes of CYP2C19*2 and CYP2C19*3 among Blacks has been reported to be from 1 to 4.3% and from 0.5 to 2.3%, respectively [12]. The frequency of the CYP2C19*2 allele was shown to be 29.7% while CYP2C19*3 was not present among North Indian population [13]. It has been indicated that the frequency of the CYP2C19*2 and CYP2C19*3 alleles were 35% and 1% among South Indian population (Tamil, Telgu, Kannada, and Malayalam) [14]. The aim of this study was to determine the CYP2C19 genotype profiles of Baluch ethnic group by studying allelic differences and compare their frequencies with results of other studies in different populations.

MATERIALS AND METHODS

One hundred and forty unrelated healthy Baluch origin people (people who speak Baluch as a native language and population inbreeding people) who were referred to Health Center in Chahbahar, in Sistan and Baluchestan province (located in South East of Iran) enrolled in the study. Five milliliters venous blood was obtained from each subject and collected into EDTA tubes. Isolation of DNA was done from peripheral leukocytes by salting out method [15]. Isolated DNA after dissolvation in sterilized distilled water and samples were stored in -20 ^{o}C. Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique were used for Genotyping of CYP2C19 alleles (CYP2C19*1, CYP2C19*2, and CYP2C19*3 alleles) [16]. PCR was carried out in 25 microliter mixture containing PCR buffer 10 mM Tris–HCl, pH 9, 1.5 mM MgCl2 (Fermentase, Burlington; Canada), 50 mM KCl (Fermentase, Burlington; Canada), 10 mM deoxyribonucleotide triphosphate (dNTP) mix, 5 U/µl Taq polymerase (Fermentase, Burlington; Canada), 5 pM of each primer (Bioneer; Korea), 500 ng DNA (Genomic; Korea) and sterile distillated water. PCR was done in genetix CG palm-thermocycler (New Delhi; India). Products of PCR (10µl)

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were digested with restriction enzymes (Fermentase; Burlington; Canada) (SmaI for CYP2C19*2 and BamHI for CYP2C19*3) at 30°C and 37°C for 16 hrs for complete digestion, respectively. Amplification of primers was explained by De Morais et al.[17]. The fragments of DNA were electrophoresed (Apelex, France) on a 2% (for CYP2C19*2) and 3% (for CYP2C19*3) agarose gel. The gels were stained with Ethidium bromide. Bands were detected by a short wavelength UV transluminator (Here, bands were not shown). The detection of CYP2C19*2 mutation was done using sense primer 5'-AATTACAACCAGAGCTTGGC-3' and antisense primer 5'-TATCACTTTCCATAAAAGCAAG-3'. The detection of CYP2C19*3 was done using sense primer 5'-AAATTGTTTCCAATCATTTAGCT-3' and antisense primer 5'-ACTTCAGGGCTTGGTCAATA-3'. The PCR amplification conditions for CYP2C9*2 and CYP2C9*3 as is explained: Initial denaturation, Number of cycle(s), Denaturation, Extention and Final extention step were 94°C, 300 sec.; 37; 94°C, 60 sec.; 72°C, 30 sec. and 72°C, 300 sec., respectively. The annealing temperature and time for CYP2C9*2 and CYP2C9*3 were 58°C, 20 sec. and 56°C, 30 sec., respectively.

95% confidence intervals (95% CI) for the frequency of the variant alleles of each gene were determined in Baluch subjects. Variations in allele and genotype frequencies between Bluch ethnic group and different other population from different area were determined by Fisher exact test. The SPSS version 16.0 was used to analysis the statistical data. $P<0.05$ was considered statistical significance.

RESULTS

Table 1 is shown the allele and genotype frequencies of CYP2C19 gene among Baluch ethnic group. The allele frequency of CYP2C19*1, CYP2C19*2 and CYP2C19*3 were 88.93% (95% CI: 83.59-94.26), 10% (95% CI: 4.90-15.09) and 1.07%, respectively. CYP2C19*1 was the most frequently (88.93%) allele in Baluch ethnic group. 78.57% of subjects were with CYP2C19*1/*1 genotype (95% CI: 71.53 -85.60). 20%, 0.71% and 0.71% subjects were with CYP2C19*1/*2 (95% CI: 13.15 - 26.85), CYP2C19*1/*3 and CYP2C19*3/*3 genotypes, respectively. There were no genotypes of CYP2C19*2/*2 (0%) and CYP2C19*2/*3 (0%). Out of the alleles and genotypes, CYP2C19*1 (88.93%) and CYP2C19*1/*1 (78.57%) were the most frequently observed mutant allele and genotype in this study. Predicted phenotypes of CYP2C19 were shown in table 2. 0.71% of subjects were poor metabolisers (PM) having CYP2C19*2/*2 and CYP2C19*3/*3 genotypes (95% CI: 2.45-6.84). 20.71% of subjects were Intermediate metabolisers (IM) having CYP2C19*1/*2, CYP2C19*2/*3 and CYP2C19*1/*3 genotypes (95% CI: 10.18-18.52). 78.57% was found to be extensive metabolisers (EM) for CYP2C19*1/*1 genotype (95% CI: 61.13-77.11). EM and PM genotype frequencies were high and low (78.57% and 0.71%) in Baluch ethnic group (Table 2). Tables 3 and 4 are shown the comparison of CYP2C19 alleles and genotype and poor metabolizer frequency among Baluch and different ethnic groups.

Table 1 Genotype and allelic frequency of *CYP2C19* among Baluch ethnic group (n = 140).

Genotype	Observed frequency n (%)	95% CI	allele	Frequency (%)	95% CI
CYP2C19*1/*1	110 (78.57)	71.53 -85.60	CYP2C19*1	88.93	83.59-94.26
CYP2C19*1/*2	28 (20)	13.15 - 26.85	CYP2C19*2	10	4.90-15.09
CYP2C19*1/*3	1 (0.71)	-	CYP2C19*3	1.07	-
CYP2C19*2/*2	0 (0)	-			
CYP2C19*2/*3	0 (0)	-			
CYP2C19*3/*3	1 (0.71)	-			
Total Number	140 (100)			100	

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Table 2 Prevalence of CYP2C19 predicted phenotypes in Baluch ethnic group (*n* = 140).

Genotype	predicted phenotype	Frequency (%)	95% CI
CYP2C19*1/*1	EM	78.57	61.13-77.11
CYP2C19*1/*2, CYP2C19*1/*3 and CYP2C19*2/*3	IM	20.71	10.18-18.52
CYP2C19*2/*2 and CYP2C19*3/*3	PM	0.71	2.45-6.84

EM: Extensive metabolisers, IM: Intermediate metabolisers,
PM: poor metabolisers.

Table 4 Comparison of CYP2C19 genotype and poor metabolizer frequency among Baluch and different ethnic groups

Study groups	Total sample	Genotype frequency % (P-value versus Baluch ethnic group)						PM genotype (%)	References
		*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3		
Baluch	140	78.57	20	0.71	0	0	0.71	0.71	The present study
Russians	290	78.7	19	0.3	1.7	0.3	0	2(NS)	18
Croatians	200	73	24	0	3	0	0	3(P=0.04)	19
Swedish	83	71	27	1	1	0	0	1.2(NS)	20
Burmese	127	44.1	39.4	5.5	9.4	1.6	0	11(P<0.0001)	21
Karen	131	51.1	39.7	0.8	7.6	0.8	0	8.4(P<0.0001)	21
Malaysians	142	42	40	6	6	6.3	1	12.7(P<0.0001)	22
Jewish Israel	140	70.7	26.4	1.4	2	0	0	2.82(NS)	23
Danish	239	71.5	24.7	0	3.8	0	0	3.8(P=0.045)	24
Belgian	121	83.5	15	0	1.6	0	0	1.6(NS)	25
Ethiopians	114	75	19	1	3	3	0	5.3(P=0.03)	26
Beninese	111	74	26	0	0	0	0	0	25

Variations in the genotype frequencies were determined by Fisher exact test. NS: No significant differences.

DISCUSSION

We regarded it important to investigate the allele and genotype frequencies of some variants of CYP2C19 in this ethnic group. There are several studies on the genetic polymorphisms of CYP2C. Several studies have been shown that genetic polymorphisms of CYP2C have indicated its clinical importance in drug efficacy between different ethnic groups. In present study, we assessed the frequencies of CYP2C19 variants in the Baluch ethnic group and compared with other different ethnic groups. For CYP2C19 allelic and genetic variants, CYP2C19*1 and CYP2C19*1 /*1 are the most common variant among Baluch ethnic group. The frequencies of 88.93% and 78.57% were found in the present study. These findings were approximately in accordance with findings in other populations [18-26] (Tables 1 and 2). The frequency of CYP2C19*2(10%) was lower and higher than values reported in studies of different other populations. Its frequency was lower than Russian (11.4%), Croatians (15%), Swedish (14%), Burmese (30%), Karen (28%), Malaysians (28%), Jewish Israel (15%), Danish (16%), Ethiopians (14%) and Beninese(13%) [18-21 and 25] and higher than Belgian (9.1%) [25] (Table 2). The frequency of CYP2C19*2 is founded approximately 10% all over the worldwide which is in agreement with our study. Clinical studies of CYP2C19*2 allele showed that patients with this allele indicated lower metabolite activities, reduced platelet inhibition activity and higher rates of cardiac incidents [27]. These results show that the CYP2C19**2* mutation took place between different ethnic groups.

Table 3 Comparison of CYP2C19 alleles among Baluch and different ethnic groups.

CYP2C19 alleles	Russians (%)	Croatians (%)	Swedish (%)	Burmese(%)	Karen(%)	Malaysians(%)	Jewish Israel(%)	Danish(%)	Belgian(%)	Ethiopians(%)	Beninese(%)	The present study (Baluch) (%)
*1	88	85	85	66	71	66	84	84	90	85	87	88.93
*2	11.4 (NS)	15 (P=0.002)	14 (P<0.05)	30 (P<0.0001)	28 (P<0.0001)	28 (P<0.0001)	15 (P=0.03)	16 (P=0.03)	9.1 (NS)	14 (P=0.04)	13 (NS)	10
*3	0.3(P<0.001)	0(P<0.0001)	0.1 (P<0.0001)	4 (P=0.005)	1 (NS)	6 (P=0.002)	1 (NS)	0 (P<0.0001)	0 (P<0.0001	3 (P=0.009)	0 (P<0.0001	1.07
Total sample	290	200	83	127	131	142	140	239	121	114	111	140
References	18	19	20	21	21	22	23	24	25	26	25	-

Variations in the allele frequencies were determined by Fisher exact test. NS: No significant differences.

The frequency of *CYP2C9*3* in our study group (1.07%) was approximately similar to the frequencies found in Karen (1%) and Jewish Israel (1%) [21,23]. The frequency of the CYP2C19*3 allele was high in Baluch ethnic group when compared with Russian (0.3%), Croatians (0%), Swedish (0.1%), Danish(0%), Belgian(0%) and Beninese(0%) [18-20 and 24-25]. Its frequency was low in comparison with Burmese (4%), Malaysians (6%) and Ethiopians (3%) [21-22, 26]. These differences of alleles are shown in Table 2. These findings show the CYP2C19*2 and CYP2C19*3 mutations are different among various ethnic groups which are in collaborate with decreased enzyme activity. These differences may be collaborating with some factors such as the ethnic origin, geographical regions and environmental factors, etc. Comparison of Bluch ethnic group with other populations shows variations and similarity in the frequency of CYP2C19 allele and PM genotype (table 4). The frequency of PMs is higher in Burmese (11%) [21], Karen (8.4%) [21] and Malaysians (12.7%) [22] Populations than Baluch ethnic group (0.71%). These results mention that the prevalence of PM phenotype with respect to CYP2C19 in the Baluch ethnic group could be lower than in other populations (table 4). The frequency of CYP2C19 polymorphism variations has epidemiologic importance in different ethnic groups. Genetic background differences in CYP2C19 polymorphism cause that some drugs metabolize differently. Studies have been shown that Japanese and Caucasian give low and no respond to the drug with therapeutic dosage [29-31]. Another study has indicated that Asian populations reveal slower metabolism of diazepam than Caucasians. This may explain with existence of high frequency of the CYP2C19*2 and CYP2C19*3 alleles in Asian populations [32]. Thus, it must be concerned with the dosage of diazepam in Asian populations.

CONCLUSION

The allelic variants of CYP2C19, CYP2C19*2 and CYP2C19*3 in Baluch ethnic group are comparable to other different population. Our results show the existence of differences in the CYP2C19 allele and genotype frequencies in different ethnic groups. CYP2C19*1 was the most frequently allele (88.93%) in a subjects of 140 Baluch ethnic group. It is important to study the clinical significance of those variations for optimal drugs dosage which metabolize by the CYP2C19 enzymes in response to different substrates such as S-mephenytoin, methylphenobarbital, omeprazole, phenytoin, imipramine, proguanil, propranolol, and diazepam [33].

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