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The Frequency of CYP2C19*2 Allelic Variant in East Azerbaijan Province of Iran Healthy Volunteers

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ABSTRACT

CYP2C19 is a drug-metabolizing enzyme involved in the metabolism of a number of chemotherapeutic agents as well as cyclophosphamide. Variants of the CYP2C19 gene result in a loss of function polymorphism, which affects approximately 3% of the Caucasian population. Lessened S-me phenytoin 4-hydroxylation is a well-described genetic polymorphism disturbing drug metabolism in individuals. These individuals are poor metabolizers (PM) of a wide range of medications including omeprazole (OMP). While an indigenous difference in its distribution of polymorphism has been defined, it is not known whether there is an ethnic heterogeneity of the structure and expression of the CYP2C19 enzyme in the Iran Azeri population. Methods: Study subjects were 200 healthy, unrelated Iranian Azeri aged 27–54 years. CYP2C19 genotype was determined in 200 healthy individuals using PCR-RFLP analysis for the two important allelic variants (*2,681G>A). Results: Genotyping of CYP2C19 revealed that the prevalence of CYP2C19*2in the Iran Azeri population was 26.7%. This is significantly (p=0.03) higher than that would be predicted from the genotypic status of these cases in CYP2C19*2 allelic variants. Conclusions: There is increasing interest in using pharmacokinetics to 'individualize medicine', however, the results of this study indicate that in a healthy population genotyping for CYP2C19 would significantly underestimate the number of phenotypic PM of drugs, such as omeprazole, which may be metabolized by this enzyme.

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INTRODUCTION

Helicobacter pyloriinfection has been associated in a variety of gastric and duodenal pathologies and illnesses: fromdyspepsia, gastritis, duodenal ulcer, and gastric ulcer to gastric lymphoma and gastric adenocarcinoma [1;2]. The annihilation of bacterial infection is a real method for thetreatment and prevention of these H. pylori related circumstances [3;4].

Treatment protocols forH. Pylori annihilation contain of a proton pump inhibitor (PPI) combined with, mostusually, two antibiotics, such as amoxicillin and clarithromycin or metronidazole. PPIs are extensively metabolized in liver by CYP2C19 that demonstrates genetic polymorphism. Metabolism of PPIs and their pharmacokinetics dependon the cytochrome P450 enzymes in the liver, mostlyS-mephnytoin 4-hydroxylase, which is mediated by theCYP2C19 genotype [1;4;5]. The CYP2C19 genotype subsists inthe form of three polymorphisms with separate effects on thepharmacodynamics of PPIs. There are two CYP2C19 phenotypes and genotypes viz., extensive metabolizers (EMs; CYP2C19*1/*1 and CYP2C19*1/*Z; where 'Z' represents a mutant allele) and poor metabolizers (PMs; CYP2C19*Z/*Z) with marked inter-individual variations in the pharmacokinetics andpharmacodynamics of PPIs in the population [6;7]. Frequency of CYP2C19 PMs differs in different population [8;9].The demographic distribution of these genotypes variesamong different ethnic populations and among landmasses.In general, Asians have a significantly higher incidence ofthe promising, from the point of pharmacokinetics, PM genotype in a range of 13–23% as compared with Caucasians andAfrican Americans whose occurrence rate is in the range of2–5% [10-12]. Because the acid secretory status of the stomachcontrols the antimicrobial properties of the antibiotics, these CYP2C19-related differences in the ability of PPIsto prevent gastric acid secretion have encouraged suggestionsthat genotyping could be a valuable tool in the optimization ofH. Pylori annihilation therapy [4;13]. It is also described that the ant secretory efficacy

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of various PPIs is affected by CYP2C19 polymorphisms to different grades, with omeprazole being affected the most followed by lanso-prazole and rabeprazole being least affected[14]. Over the years, several randomized controlled trialshave been published concerning the effect of the CYP2C19genotype on various PPIs used in dual and tripleH. pylorieradication therapies consisting of amoxicillin and/or clarithromycin often with incompatible results[15]. Thus, theaim of this study is to show CYP2C19genotype frequencyin healthy population of East Azerbaijan Province of Iran.

MATERIAL AND METHODS

Venous blood samples and DNA purification:

From each case, 1 ml venous blood was collected. The 200 healthy adult volunteers had no drug administration. Informed consent was obtained from all volunteers. Samples were divided into 500µl aliquots in Eppendorf tubes, and kept at -20$^{\circ C}$ until required. Leukocyte genomic DNA was extracted directly from the blood samples using a QIAamp Blood Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol.

*PCR amplification of CYP2C19*2 region:*

GenomicDNA (150 ng) was amplified in 2X PCRMaster Mix (0.2 U/µl Taq DNA polymerase; 0.4mMdATP, dCTP, dGTP, and dTTP; 2.0 mM MgCl2; Tris-HCl pH 8.5, (NH4)2S04, 4mM MgCl2, 0.2% Tween®20; Ampliqon, Denmark) containing nuclease-free water in a total volume of 20µl, with PCR primers at a concentration of 0.2µM. A previously reported PCR primer set for CYP2C19*2[16]. Amplification of this region was performed with aMultigeneOPTImaxLabnet PCR system (Labnet, USA) using an initial denaturation step of 94 ◦C for 10 min; 32 cyclesof 94◦C for 30 s, 59◦C for 30 s, and 72◦C for 50s.The amplified PCR products (167 bp for the CYP2C19*2 region) was analyzed on a 3% agarose gel with a 50 bp ladder (Fermentase, Carlsbad, CA) as a molecular weight marker (Fig 1).

Restriction enzyme digestion of PCR product:

To detect the CYP2C19*2 defect, 10µl PCR product was digested with 0.5 U SmaI (Fermentase, Carlsbad, CA) in a complemented reaction buffer in a total volume of 20 µl at 37◦C for 18 h. Digested product was analyzed on a 12% polyacrylamide gel. The wild type appears as two bands of digestion products (117 and 50 bp for CYP2C19*2) On the other hand, the homozygous mutated type appears as a single band of undigested product (167 bp for CYP2C19*2). If all products (undigested and digested) appeared on the gel, the subject was a heterozygote.

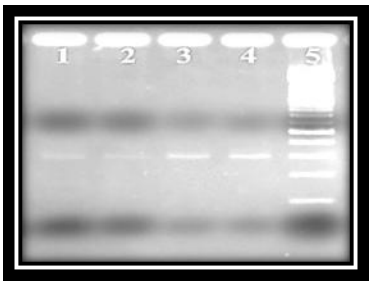

Fig. 1: Lines 1 to 4 shows 167 bp for the CYP2C19*2 region and 5th line 50 bp ladder, respectively.

Table 1: Demographic characteristics of the subjects.

	Characteristics of Samples		
Age, mean (SD), y	44 (2.1)		
Men, % of patients	53		
women, % of patients	47		
Smoking status (%)	49		
Body mass index (kg/m2)	28.6+/- 0.1		
Omeprazole usage history (%)	84.2		
Family history of Duodenal ulcer (%)	14.8		
Helicobacter Pylori serologic test (%)	Positive for IgM	Positive for IgG	Negative
	11.3	76.4	12.3

Results:

The subjects' sex, age, weight and smoking status are presented in Table 1.In the wild type gene, cleavage of PCR products yields fragments of 117 and 50 bp for CYP2C19*2. With individuals homozygous for CYP2C19*2, the SmaI site in exon 5 is destroyed and the 167 bp fragment is not cut (Fig 2).With heterozygous individuals, all three bands (50, 117, and 167 bp for CYP2C19*2) are evident. Allele frequencies of CYP2C19*2 was 0.267 (107 of the total of 400 alleles).

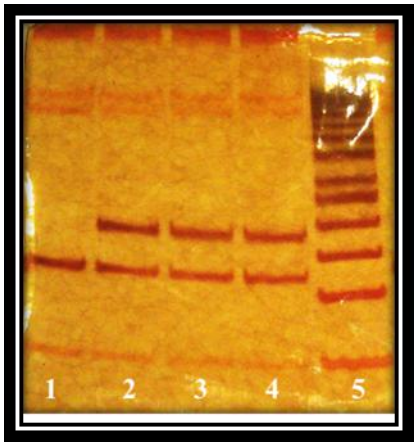

Fig. 3: Lines 2 to 4 showsall three bands (50, 117, and 167 bp for CYP2C19*2, Heterozygote), line1 shows two bands (50, 117 for CYP2C19*2, no mutation) and 5th line 50 bp ladder, respectively.

Discussion:

CYP2C19 plays an important role in the metabolism of many types of drug. Polymorphisms in CYP2C19 are known to affect the metabolic activity of some commonly used drugs, such as omeprazole, proguanil, and certain tricyclic antidepressants. If a CYP2C19 polymorphism can be identified before medication, the most appropriate drug regime can be resolute for each individual [17-20]. A modified PCR-RFLP technique was used to determine the details of CYP2C19 polymorphism in East Azerbaijan Province of Iran. The PCR-RFLP method is a rapid and simple technique for detecting genetic defects [21]. Zalloum reported that all Japanese EMs have at least one wild-type allele, and that CYP2C19m1(CYP2C19*2) and CYP2C19m2 (CYP2C19*3) are allelic and segregate as two independent mutated alleles[22]. This indicates that it is possible for CYP 2C19*2 and CYP 2C19*3 to exist on the same allele.Our results warn of a drawback of the PCR-RFLP method [14;23;24]. In recent years, many population studies have used only PCR-RFLP. Although PCR-RFLP is a simple and very useful screening method, mutations should be confirmed by sequence analysis, especially in compound heterozygotes. PPIs play a critical role in facilitating the efficacy of antimicrobial agents and, therefore, have been considered key to determining high H. pylori eradication rates [25;26]. A number of reviews have presented contradictory evidence demanding evidence for or against the influence of PPI-related CYP2C19 polymorphisms on eradication rates.The frequency of occurrence of PMs in north Indians was intermediate as that of Caucasians (1-7%) and Orientals (15-23%)[27;28]. Since the occurrence of PMs was same in the normal healthy volunteers in the present study, it indicated that possessing PM trait is not a risk factor for gastritis in north Indians. However, a few studies reported a correlation between CYP2C19 PM Trait and gastric cancer.In conclusion, results of the present study demonstrated that CYP2C19 genetic polymorphism is an important determinant of the efficacy of PPI based anti H. pylori therapy in East Azerbaijan Province of Iran. If CYP2C19 phenotype of a patient is known prior to therapy, an optimal dose of the PPI can be prescribed to achieve better management of patients having H. pylori associated gastritis, gastric ulcers and duodenal ulcers.

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