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Allele frequency of *CYP2C19* (**1* and**3*) gene polymorphism in Palestinian population

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Abstract
Human cytochrome P450, particularly CYP2C19 play an important role in drug metabolism. The polymorphisms of this enzyme give rise to change variability in drug excretion rates and function. In this study we determined genotypes of *CYP2C19* (**1* and **3*) in Palestinian population to compare allele frequencies with other ethnic groups finding. CYP2C19 (*1/*3) allelic variants were determined in 213 unrelated healthy Palestinian volunteers by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. Three subjects (1.4%) were homozygous for CYP2C19*3, 88 (41.3%) were homozygous for wild type allele (*CYP2C19*1/*1*) while 122 (57.3%) individuals were heterozygous for variant allele (*CYP2C19*1/*3*). The frequency of *CYP2C19*1* and *CYP2C19*3* alleles were 0.692 and 0.308 respectively. In addition, based on our data, the frequencies of EM and PM were 98.6 % and 1.4% respectively. Further studies are required to evaluate the metabolic and clinical significance of other *CYP2C19* alleles, in the Palestinian population.

Keywords: CYP2C19*3 polymorphism, Palestinian population, allele frequency, extensive metabolizer, poor metabolizer

1. Introduction
Cytochrome P450 (CYP) superfamily enzymes metabolize a wide range of clinically significant drugs, as well as other xenobiotics. The related CYP2C19 (Cytochrome P450, family 2, subfamily C, polypeptide 19) gene is located on chromosome 10q 23. 33 [1]. The CYP2C19 gene has nine exons and spanning approximately 90 kb and produces a protein of 490 amino acids, is highly polymorphic, with over 25 variant star (*) alleles currently defined by the Human Cytochrome P450 Allele Nomenclature Committee (http://www.cypalleles.ki.se/CYP2C19.htm).
The highly polymorphic *CYP2C19* gene has at least 22 identified allelic variants [2, 3] and although some of these allelic variants produce enzymes with decreased or absent function. Of these allelic variants, only 4 (*CYP2C19*1*, **2*, **3*, and **17*) are of significant allelic frequency generally identified in the vast majority of the human population [4]. CYP2C19, a member of the cytochrome P450, is involved in the biotransformation of important drugs, such as: Proton Pump Inhibitors (PPIs) [5-7] Anticonvulsants (S-mephenytoin and diazepam) [8, 9]. CYP2C19 metabolizes several therapeutically important drugs, namely omeprazole, lansoprozole, propranolol, imipramine, mephenytoin, chloroguanide, hexabarbitone and diazepam [10].
The drug metabolism is directly related to genetic polymorphism and gene mutations influence the enzyme activities responsible for the drugs metabolism [11-13]. On the basis of *CYP2C19* genotypes, (on the basis of their ability to metabolize (S)-mephenytoin or other CYP2C19 substrates)individuals can be grouped into poor metabolizer genotypes (PMs, the sum of homozygous and heterozygous genotypes of *CYP2C19*2/*2*, **3/*3* and **2/*3*) and extensive metabolizer genotypes (EMs, *CYP2C19*1/*1*, **1/*2* and **1/*3*) [14]. CYP2C19 PMs may suffer unwanted adverse effects with a normal dose of a drug inactivated by CYP2C19, and may also show weak responses to drugs that need to be activated by CYP2C19 [14].
*CYP2C19*3* arises from a G→ A transition at position 636 in exon 4 of *CYP2C19*, which produces a truncated protein, that results in a premature termination codon at amino acid 212 [15] changes the tryptophan codon to the termination codon, which leads to protein synthesis stopping earlier and the protein become functional defect [16]. The *CYP2C19*3* allele frequencies in most populations are below 1%; however, it is more prevalent among Asians (2–9%) [3].
The aim of this study was to genotype healthy Palestinian people for cytochrome *P4502C19*, to identify the variant allele of *CYP2C19* (*CYP2C19*3*) at position 636 in exon 4 of

*CYP2C19*and to compare the results with findings in other countries.

2. Methodology

2.1. *Sample collection*

Venous blood samples were obtained from healthy unrelated Palestinian volunteer (n=213).The ethical approval of the study was obtained, a written informed consents were obtained from all participants. 2.5 ml of venous blood was collected in EDTA tubes from each volunteer. The EDTA sample kept at 4 °C was used within 24h for DNA extraction and subsequent PCR analysis.

2.2 DNA extraction and genotyping

DNA extraction was performed using Wizard DNA extraction kit (Promega, USA) following the manufacturer's instructions from fresh EDTA whole blood cells and stored at -20ºC until PCR analysis. The quality of the isolated DNA was determined by running 5 µl of each sample on ethidium bromide stained 1.0% agarose gels and the DNA was visualized on a short wave U.V. transilluminator.

PCR was performed using the primers described by Morais *et al* [17, 18]. A 271bp fragment was amplified from genomic extracted DNA using the following oligonucleotide primers:

forward: 5' TATTATTATCTGTTAACTAATATGA 3' and

reverse: 5' ACTTCAGGGCTTGGTCAATA 3'

3µl (~150ng) of prepared DNA template was added to 20 µl master mix (Bioline, UK), and 1.5 µl of each primer (5pmol) in 0.2 ml thin walled microfuge tube. PCR was performed in a thermal cycler (Biometra, Germany). The cycling conditions were: an initial denaturation for 5 min at 95 °C, followed by 35 cycles of 20s at 94 °C, 15s at 48 °C, 10s at 72 °C and an additional 10 min at 72 °C for final extension. The PCR product were detected on ethidium bromide stained 2% agarose gels.

2.3 Restriction Fragment Length Polymorphism Analysis

Genotyping for *CYP2C19* was performed by digesting the PCR product with restriction enzymes (BamHI) and separation of the resulting DNA fragments on 3% agarose gels. For RFLP analysis the following mixtures were prepared in clean two 0.2ml microfuge tubes.

For RFLP analysis, a mixture of: 10 µL PCR product, nuclease-free water 34 µL, 5 µL of 10X Buffer and 1µL of BamHI (20,000 U/ml) added in a clean 0.2 ml microfuge tube. The mixture had been mixed gently and spin down for a few seconds. The contents were incubated at 37 °C for 15 min. The digests were resolved on 3% ethidium bromide stained agarose gels and the results were documented by photography.

Digestion of the fragment with BamHI yielded an undigested band of 271 bp from the *A* allele (abnormal allele) or fragments of 175 and 96 bp with the *G* allele (normal allele). Since the restriction site is absent in the mutant alleles, the PCR products are not digested by restriction enzymes. In the *CYP2C19* (wild-type) allele the restriction enzymes BamHI splice the 271-bp DNA fragments into 175 bp, 96 bp.

The *CYP2C19* homozygote should yield one band (271 bp) while the *CYP2C19* heterozygotes should produce three bands (271 bp, 175 bp, and 96 bp).

The observed genotype frequencies of CYP2C19*3 were compared with expected genotype frequencies according to the Hardy–Weinberg law.

3. Results

3.1 Study Population

The study population consisted of 213 samples (126 male, 87 female). The mean age of subjects was 53.95± 11.53. The percentage of males was 59.2% while that of females was 40.8%. 70.4% of the participants were non-smokers and 29.6% were smokers.

3.2 PCR Results

3.2.1 PCR amplification of the CYP2C19*3

The PCR product generated from the *CYP2C19*3* polymorphism should yield a 271 bp-long double stranded DNA fragment. A negative control (with water instead of the DNA template) was included in each reaction. The size of the amplicon was estimated by comparing it with a DNA molecular size marker (50 bp ladder DNA) run on the same gel.

3.3 RFLP Result and Genotype Frequencies

Among 213 subjects tested, 88 were homozygous for wild type allele (*CYP2C19*1/*1*), 122 individuals were heterozygous for variant allele (*CYP2C19*1/*3*) and 3 subjects were homozygote for variant allele (*CYP2C19*3/*3*).The *CYP2C19* allele frequencies in the study population were 30.8% for the **3* allele and 69.2% for the **1* allele. The distribution of the subjects according to *CYP2C19* genotypes were as follows, **1/*1*(*G/G*): 41.3%, **1/*3*(*G/A*): 57.3%, and**3/*3*(*A/A*): 1.4%.

There were no significant differences in allele distribution between the male and female subjects in all the participants. Based on Hardy–Weinberg equilibrium, no significant differences in the expected and observed frequencies of the three genotypes exist. Regarding phenotype the frequencies of EM and PM were 98.6 % and 1.4% respectively

4. Discussion

The CYPs are the main drug metabolizing enzymes in human body and the CYP2C19 is one of the most important enzymes in this large family of proteins. For the *CYP2C19* which is a highly polymorphic locus there is currently 27 alleles, only 3 of which are with normal activity and one with high activity, the others are with no enzymatic activity [19]. The polymorphism of *CYP2C19* has been shown to have the most ethnic variation with PM frequency ranges from 2 to 7 % in Caucasians, to 14–25 % in Asians [20]. We estimated the distribution of *CYP2C19* in the Palestinian population and compared these data with those from other populations.

In the present study, we found that the incidence of *CYP2C19*3* homozygous genotype in 3 Palestinian subject (1.4%) which was consistent with other populations (Table 1). Furthermore, this study shown that the frequency of *CYP2C19*3* allele was 0.308%. Nevertheless there is limited numbers of studies have reported *CYP2C19*3* in populations other than the Asians. *CYP2C19*3* was reported in the Egyptian population 0.06% [20], Lebanese population 0.03% [23], Turkish 0.01% [33], Iraqi 0.2% [21], Iranian(Turkman) 0.2% [26], Malaysians 0.06% [28], Koreans 0.116% [20] and Chinese 0.045% [9]. While *CYP2C19*3* has not been reported in populations like: Jordanian [24], Iranian (Tehran) [25], American [30] and Italians [32]. The frequency of *CYP2C19*1* allele was 0.692 in Palestinian subjects that shown by present study. Similar results were obtained by Strom *et al*. they reported that the *CYP2C19*1* allele frequency in: White, African American, Hispanic/ Latino and Ashkenazi Jewish

were: 0.66, 0.63, 0.75 and 0.70 respectively [34].
According to our result the EM phenotype (**1/*1*: 41.3% and **1/*3*: 57.3%) in 213 Palestinian subjects was 98.6% while the frequency of PM was 1.4%. Similar results were obtained by Strom *et al*; they reported that the EM due to CYP2C19*1 homozygous (**1/*1*) were: 42%, 39% and 46% for White, African American and Ashkenazi Jewish respectively [36]. Sahib *et al*. also reported that the EM due to CYP2C19*1 homozygous (**1/*1*) in Iraqi population was 43.9% [21].
In addition the result of this study was similar to that obtained in Chinese population. The metabolic rate Chinese population include the following: 43.7% of populations were extensive metabolizer (**1/*1*) and 1% as poor metabolizer (**3/*3*) [35].
In Asian populations the frequencies of poor metabolizer was higher than that observed in this study. In Thai population the frequencies of poor metabolizer was 13.03% [36]. In Korean population the frequency of poor metabolizer was 12% [37]. This can be explain higher frequencies of CYP2C19*3 in these population [30, 38].

Table 1: Comparison of allele frequencies of *CYP2C19* (**1* and **3*) reported from different ethnic populations

Population	No.	Frequency of *CYP2C19*		References
		1	***3***	
Palestinian/Gaza Strip	213	0.692	0.308	Present study
Iraqi	221	0.651	0.2	[21]
Egyptians	494	0.888	0.002	[20]
Saudi Arabia	97	0.628	NA	[22]
Lebanese	161	0.863	0. 03	[23]
Jordanian	78	0.84	0.0	[24]
Iranian: Tehran	200	0.86	0	[25]
Iranian: Turkman	140	0.564	0.2	[26]
Tunisian	100	0.885	NA	[27]
Malaysians	142	0.66	0.06	[28]
Japanese	265	0.708	NA	[29]
Koreans	103	0.675	0.116	[20]
Chinese	121	0.495	0.045	[9]
American	100	0.80	0.0	[30]
Turkish	100	0.86	0.01	[31]
Italians	360	0.889	0.0	[32]
Greek	283	0.794	NA	[33]

NA: not analyzed

5. Conclusion
In conclusion, the results of this study suggest a relatively high allelic frequencies of *CYP2C19*3* in Palestinian population. The data also indicate either high frequency of screened EM (*CYP2C19*1/*3*) in the studied volunteers. Future work is required to evaluate the metabolic and clinical relevance of other CYP2C19 alleles, in the Palestinian population

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