CYP2C19 GENOTYPES IN A POPULATION OF HEALTHY VOLUNTEERS AND IN CHILDREN WITH HEMATOLOGICAL MALIGNANCIES IN GAZA STRIP

Abu-Eid I Sameer[1], Gharbieh M Amany[2], Abed A Abdela[3], Sharif A Fadel[4]

[1]European Gaza Hospital, Laboratory Department; [2]Biological Science, Masters Program; [3]Biology Department, IUG-GAZA; [4]Medical Technology Department, IUG- Gaza, Palestinian Authority

Corresponding Author: *sameer_eid@yahoo.com*

ABSTRACT

Background
Cytochrome P450 2C19 (CYP2C19) participates in the metabolism of many clinically important drugs and xenobiotic compounds. Genetic polymorphisms of the CYP2C19 gene are described to have possible effect on drug treatment and increasing susceptibility to carcinogenic substances. The aim of this study was to determine the frequencies of the common polymorphic CYP2C19 alleles (CYP2C19*2 and CYP2C19*3) in Gaza Strip population and to investigate their association with occurrence of childhood hematological malignancies as compared to healthy subjects.

Methods
The polymorphism of CYP2C19 was analyzed by PCR-RFLP. DNA was extracted from blood samples obtained from 52 previously diagnosed hematological malignancy children and 200 normal subjects.

Results
In the patient group the frequencies of CYP2C19*2 and CYP2C19*3 were 9.62% and 0.96%, respectively; while in the control group the respective frequencies were 5.75% and 3%. There is no significant difference between the healthy and the patient groups in terms of the frequencies of CYP2C19*2 and CYP2C19*3. The genotyping analysis showed the following results: 15.39% (1*/2*), 1.92% (1*/3*), 1.92% (2*/2*) and 80.77% (1*/1*) in the patients, while in the normal subjects the distribution of CYP2C19*1/*1, *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 genotypes were 86.5, 6.5, 3, 1.5, 2, and 0.5 %, respectively.

Conclusion
There is no significant association between the CYP2C19 polymorphism and the occurrence of the childhood hematological malignancies. The distribution of CYP2C19*2 in the Gaza Strip population is lower than that in Caucasians, Africans and the Asian populations. The CYP2C19*3 allele, which was not reported in the Caucasian populations, is present in 3% of the Gaza Strip population. Further studies are needed to investigate the role of other CYPs' polymorphisms in our patient group.

Keywords: *CYP2C19, polymorphism, hematological malignancy, PCR-RFLP, Gaza Strip*

Cytochrome P450 (CYP) proteins are heme enzymes that function in diverse pathways, from carbon source assimilation to hormone biosynthesis.[1] Humans have been estimated to have at least 57 different CYP genes and 47 pseudogenes[2], but the major drug metabolizing human P450s are CYP1A, CYP2B, CYP2C, CYP2D, CYP2E and CYP3A.[3,4,5] The human CYP2C subfamily consists of four members (CYP2C8, -9, -18 and -19), which share >82% amino acid identity.[6] The CYP2C19 gene is located within a cluster of CYP genes on the 4th band of region 2 of the long arm of chromosome 10 (10q24).[7] CYP2C19 is responsible for the

metabolism of a number of therapeutic agents such as the anticonvulsant drug S-mephenytoin, omeprazole, proguanil, certain barbiturates, diazepam, propranolol, citalopram and imipramine.[8] Drugs may be themselves substrates for CYP2C19 enzyme and/or may inhibit or induce the enzyme.[9]

About 24 variants of CYP2C19 are known. The most important of these alleles are: CYP2C19*2 (681G→A) and CYP2C19*3 (636G→A). The nucleotide changes in the CYP2C19*2 and *3, lead to a splicing defect and stop codon, respectively, and therefore to nonfunctional proteins, hence the name poor metabolizer (PM) phenotype.[10,11] They represent more than 99% of all the abnormal CYP2C19 alleles in Asian population and 87% in Caucasian population. The PM phenotype has been shown to represent 13 to 23% of Asian populations, but approximately 2 to 5% of Caucasian populations.[12,13,14]

To the best of the author's knowledge the association between CYP2C19 polymorphism and hematological malignancy has not been studied before. Many authors, however, tackled the association between the CYP enzymes and other types of cancers e.g., esophagus, stomach, lung and bladder cancers.[15]

The Haematological Malignancies are a group of neoplasms that arise through malignant transformation of bone marrow derived cells.[16] The etiology of hematological malignancies is largely unknown. Biological and epidemiological data implicate an important role of exogenous toxicants including cytotoxic drugs, benzene, radiation and tobacco smoking. Most of these substances are detoxified by CYP enzymes, which are present mainly in the liver cells.[17]

The aims of this study were to determine the allelic and genotypic frequencies of the common allelic variants for CYP2C19 in the Gaza strip population, and to investigate the association between these variants and occurrence of childhood hematological malignancies.

METHODS

Study Population

The present study was carried out on 52 children (49 ALL, 2 Lymphoma and 1 CML) from El-Nasser hospital (45 patients) and the European Gaza hospital (7 patients) and 200 unrelated healthy Palestinian volunteers residing in Gaza Strip. The control subjects were selected regardless of sex or age. All subjects were informed about the contents and aims of the study and gave their written consent. When the participants were less than 18 years old, their parents gave the written consent. The local ethics committee (Palestinian National Authority - MOH - Helsinki Committee) approved the study protocol.

Blood Samples

About 2.0 ml of venous blood were drawn into sterile EDTA tubes. The blood samples were then stored at -70°C until the time of DNA isolation.

Genotyping

Genomic DNA was isolated from 300 µl whole blood using Wizard Genomic DNA Purification Kit (Promega, USA). PCR-RFLP was performed to genotype CYP2C19*2 and CYP2C19*3. Sma I and BamH l were used as restriction enzymes for CYP2C19*2 and CYP2C19*3, respectively. The resulting DNA fragments of PCR and PCR-RFLP were analyzed on ethidium bromide stained agarose gels as described by Tamminga et.al.[18]

Statistical Analysis

Relations between CYP2C19 alleles, occurrence of hematological malignancy and comparisons of genotypes between patients and controls were performed by means of the odds ratio and the Chi-square (χ^2) test or Yates corrected Chi-square in the case of expected frequencies less than 10 or the Fisher's exact test in the case of expected frequencies less than 5. All the statistical tests were two sided; a p value of <0.05 was considered statistically significant. Multivariate analysis, correlation coefficient, odds ratios, p value (two-sided tests) and 95% CI were used to describe the strength of association.

RESULTS

CYP2C19 genotype and allele frequencies for patients and control groups are given in Tables 1 and 2, respectively. The allele frequencies in both groups showed relatively similar distributions. The frequency of CYP2C19*2 in the control group versus the patients group was not significantly different ($p > 0.05$). Moreover, the

frequency of CYP2C19*3 allele in the control (which is higher than that in patients), showed no significant difference ($p > 0.05$).

Regarding the metabolism phenotypes associated with CYP2C19 polymorphism, Table 3 illustrates a comparison between the patients and the control subjects (2*/2*, 3*/3* and 2*/3* were considered poor metabolizer "PM", while the other genotypes were considered extensive metabolizer "EM").

TABLE 1 CYP2C19 genotypes and derived allele frequencies in the patients

Genotype	n	Observed frequency	Allele	Derived allele frequency
CYP2C19*1/*1	42	80.8%		
CYP2C19*1/*2	8	15.4%	CYP2C19*1	89.4%
CYP2C19*2/*2	1	1.9%	CYP2C19*2	9.6%
CYP2C19*1/*3	1	1.9%	CYP2C19*3	1.0%
CYP2C19*3/*3	0	0%		
CYP2C19*2/*3	0	0%		
Total	52	100%		100%

TABLE 2 CYP2C19 genotypes and derived allele frequencies in the control group

Genotype	n	Observed frequency	Allele	Derived allele frequency
CYP2C19*1/*1	173	86.5		
CYP2C19*1/*2	13	6.5	CYP2C19*1	91.3%
CYP2C19*2/*2	6	3	CYP2C19*2	5.8
CYP2C19*1/*3	3	1.5	CYP2C19*3	3%
CYP2C19*3/*3	4	2		
CYP2C19*2/*3	1	0.5		
Total	200	100		100%

TABLE 3 Comparison between the patients and the control subjects in terms of phenotypic distribution

Group	PM*	EM+	P value
Patients	1	51	0.47
Controls	11	189	
Total	12	140	

*, Poor Metabolizer.
+, Extensive Metabolizer.

DISCUSSION

Investigating the association between CYP2C19 polymorphism and cancers have important implications since cytochromes are actively involved in drug, carcinogen and procarcinogen metabolism. Some are involved in the activation of procarcinogens, while others may take part in the inactivation of carcinogens. This depends on the kind of carcinogen, cancer and on the type of mechanism of carcinogenesis.[15]

The CYPs are the main drug metabolizing enzymes in the human body and the CYP2C19 is one of the most important enzyme systems in this large family of proteins. The CYP2C19 has also been shown to be responsible for the metabolism of many drugs, some of which are used in treatment of the hematological malignancies such as cyclophosphamide and glucocorticoids.[12,10]

Some investigators have shown associations between the CYP2C19 polymorphism and certain types of cancers (e.g., esophagus, stomach, lung and bladder cancers).[15] Other authors, however, did not find an association between CYP2C19 polymorphism and other types of cancers. For example, Wadelius et.al[19] evaluated the association of the variant m1 allele (CYP2C19*2) of the CYP2C19 gene with prostate cancer among a Swedish population, but no significant differences were found between cancer patients and controls. In addition, Bartsch et al couldn't find an association between the PM genotype in Japanese patients with bladder cancer, but they did find a significant association between the PM genotype and squamous cell carcinoma of the lung.[20] Our hypothesis postulated that heterozygosity and homozygosity for the base pair substitution in exon 5 (681G→A) i.e., allele 2* and exon 4 (636 G→A) i.e., allele 3* of CYP2C19 could be associated with a functional decrease in the amount of xenobiotic monooxygenase to activity. Therefore, those individuals would have a markedly increased susceptibility to the genotoxic and leukemogenic effects of cytotoxic and xenobiotic substances that they normally encounter in their daily life.

Statistical analysis of the frequencies of PMs in the hematological malignancy and the control groups showed no significant difference. However, this doesn't exclude the presence of other predisposing genetic factors (e.g., other CYPs' polymorphisms) to the hematological malignancies that may be involved in the occurrence and progression of the cancer.

A comparison of the distribution of CYP2C19 variant alleles among the Palestinian subjects and different ethnic groups presented in the literature is presented in Table 4. By comparing our results with our neighbor, the Egyptian population, we found that the incidence of CYP2C19*2 in Egyptians (11%) was similar to that found in other Caucasian populations (11–16%), but the incidence of this allele in Gaza strip population is lower (5.8%). They also reported only an (0.2%) incidence of CYP2C19*3 as compared to our finding of (3%).[21] The observed presence of CYP2C19*3 in one Gaza Strip subject was unanticipated and may be hypothesized to be the result of emigration and possible genetic admixture between the Gaza Strip population and Asian population.

TABLE 4 Distribution of CYP2C19 variant alleles among different ethnic groups

Ethnicity	Allele frequency (%)			References
	WT (*1) *	m1 (*2)†	m2 (*3)+	
Non-Asians				
Palestinian (Gaza Strip)	91.3	5.8	3	**Present study**
Egyptian	88.8	11	0.2	**[21]**
Israeli Jewish	84	15	1	**[22]**
Caucasians				
Iranian	86	14	0	**[23]**
Saudi Arabian	85	15	0	**[24]**
Australian	85	15	0	**[25]**
Canadian (Inuit)	89	11	0	**[26]**
Swedish	84.9	14.4	0.7	**[27]**
Danish	84	16	0	**[28]**
European American	87	13	0	**[29, 24]**
Asians				
Sum for Asians	62	32	6	**[29, 24]**
Chinese-Taiwanese	63	32	5	**[24]**
Japanese	67	23	10	**[29, 24]**
Filipinos	54	39	7	**[29, 24]**
Korean	67	21	12	**[30]**
South-west Asia				
North Indian	70	30	0	**[31]**
Africans				
African American	75	25	0	**[29, 24]**
Bantu-Tanzanian	81.5	18	0.5	**[30]**
Ethiopian	85	13	2	**[32]**
Venda	78	22	0	**[33]**
Zimbabwean	87	13	0	**[33]**

*, WT (*1): Wild type allele (CYP2C19*1) †, m1 (*2): CYP2C19*2+, m2 (*3): CYP2C19*3

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