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An association and genetic polymorphisms of *CYP2D6* gene in chronic renal failure patients in AL- Qadisiya province / Iraq.

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Abstract

The gene *CYP2D6* is one of the most important genes of phase I drug metabolizing enzymes. Individuals differ in response to drugs caused by mutation in *CYP2D6* gene. The patients with chronic renal failure disease (CRF) are often treated with more than one drug. This study was designed to investigate the distribution of *CYP2D6* polymorphism in patients with CRF and also to evaluate the role of this polymorphism gene as a genetic risk modifier in the etiology of CRF disease.

The study was carried out on (60) patients with CRF and (60) healthy volunteers . The *CYP2D6* genotypes were analyzed by (PCR- RFLP) method.

The results indicated that the levels of creatin and urea were significantly higher ($P < 0.05$) in CRF patients compared to control. While the mean Hb concentration, PCV level were significantly lower($P < 0.05$) in CRF patients compared with healthy subjects. There was no significant association between CRF risk and *CYP2D6* (HEM) genotype. The nonfunctional *CYP2D6* (PM) genotype had a (1.89) fold increase of risk toward CRF disease the (95 % CI= 0.18-12.32). This study showed the protective *CYP2D6* (EM) in CRF severity.

The statistical analysis showed no significant association between the age and *CYP2D6* (HEM, EM)genotypes.

The risk for developing CRF disease increased in case of heavy smokers of (1.6) fold as compared to light smokers (OR= 1.2).

Introduction

Chronic renal failure (CRF) is a common clinical syndrome characterized by decline inglomerular filtration, perturbation of extracellular fluid volume, electrolyte and acid base homeostasis and retention of nitrogenous waste from protein catabolism (1).

Patient with (CRF) are often treated with more than one drug . effects of these drugs depend on the extent of drug absorption from the gut lumen, on metabolism of the drug in the liver, and on the extent of its transport back into the systemic circulation for extra hepatic effects (2). Drug metabolizing enzymes (DME), which include phase I and II metabolizing enzymes play a central role in the intestinal absorption, metabolism elimination, and detoxification of various drugs (3). Cytochrome P450 (CYP) enzymes are major phase 1 metabolising enzymes (3). The cytochrome P450 (CYP) isoenzymes are a super family genes contains 57 functional genes (4). The 1 to 3 families of CYP are responsible for 70% to 80% of all phase I dependent metabolism of clinically used drugs (5) *CYP2D6* isoform metatabolizes more than 25% of most common drugs (6) .

CYP2D6 gene is extremely polymorphic, and more than 70 allelic variants have been described (7,8) as a result, metabolism and excretion rates of drugs vary between individuals, Different phenotypes can be distinguished poor metabolites (PM) lack the functional enzyme , intermediate metabolizes (IM) carry 2 different alleles, leading to partial activity, efficient metabolites (EM) have 2 normal alleles; efficient intermediate metabolites (EIM) are heterozygous for 1 deficient allele and ultra- rapid metabolizes (UM) have multiple gene copies (9).

The aim of this study is to investigate the distribution of *CYP2D6* polymorphism in patients with CRF and also to evaluate the role of this polymorphism gene as a genetic risk modifier in the etiology of CRF disease.

Materials and methods

Subject:

In this study we have analysed *CYP2D6* polymorphisms in a group of (60) patients diagnosed with chronic renal disease in AL-Diwanya general hospital and (60) matching health volunteers, who served as control group . They signed an informed consent and information was obtained by a standardized questionnaire , including data age, gender, disease duration, time on dialysis, another disease. The data then were statistically analyzed.

The blood sample were collected from the CRF patients and divided in to two tubes (with and without anticoagulant- EDAT)

Blood sample were collected by EDTA divided into two part , the first stored at -20 C^o till used for DNA extraction, Second part of blood samples were taken from each subject for measurement of hematological parameters including determination of hemoglobin (Hb) concentration, packed cell volume (PCV) were determined as described by (10).

The blood in non- EDTA tubes was centrifuged at 2000 rpm for 20 minutes, The clear supernatants serum were frozen till the time of biochemical estimations including the levels of Creatin and Urea, these were measured using an automatic analyzer (Reflotron) (Germany).

DNA extraction :

Two milliliter venous blood samples drawn into EDTA were obtained from each subject and genomic DNA was isolated by using Mini kit from (Genedia company) and stored directly at 4 °C till used .

PCR , RFLP Analysis of *CYP2D6* polymorphism:

The polymorphisms of *CYP2D6* analyzed by a polymerase chain reaction (PCR) techniques based on the method described by (11)

Genomic DNA (100ng) was used as a DNA template in (25 µl) of total volume reaction . The following primer were used.

CYP2D6 : F-5 GCC TCC GCC AAC CAC TCC G:3

R-5 AAA TCC TGC TCT TCC GAG GC:3

The amplification reactions were carried out in volume of 25 µl containing of primer, 1U of taq DNA polymerase, 200 mm of each dNTP, 50 mM KCl 1.5 mM . MgCl2, 10 mM Tris- HCl , and 100 – 200 ng of DNA.

After an initial denaturation at 95 C for 6 min 30 cycle were performed consisting of (94 C for 1 min, 57 C for 1 min , 72 X for 1 min and 5 min find extension for last cycle. This yielded a 334 bp fragment.

Digestion :

10µL PCR product was digested for overnight at 37 °C with *Bstn1* restriction enzyme using 2µL , 18 µL deonised water and 2 µL of R1buffer.

The digestion products were classified as Homozygous extensive metabolizer (EM) (230 – 104 bp). Heterozygous extensive metabolizer (HE-M) (334 – 230 – 104 bp) and poor metabolizer (PM) (334 bp) alleles(12) .

Statistical Analysis :

X^2 tests were used to examine differences of allele and genotype frequencies between patients and controls. Fisher's exact test was used. ORS and The 95 % CI were calculated and $P< 0.05$ considered signification (SPSS software version 14).

Results

The study subjects (60) patients (76.66% male , 23.33% females) with chronic renal failure disease and (60) healthy volunteers, who served as controls for the physiological and genetic characterization.

The mean age of patients was (42.183± 1.790) rang (20 to 70 Years), While control, it was (43.18±1.79) rang (20 to 70 Years).

No significant difference in the gender distribution was observed between cases and controls (Table 1). The table also shows the frequency match of smoking status between cases and control (43.33% and 30%) respectively. The cases and controls were matched in a higher value of smoking index (heavy smokers with a Brinkman index, BI > 500), (38.46%)of the cases and (50%) of controls.

The hemoglobin concentration and PCV were significantly lower ($P < 0.05$) in patients CRF compared to those of controls. Shown in table (1).

As many as the values of serum creatine and urea in CRF patient group comparison with control values, the levels of creatine and urea were significantly increase ($P< 0.05$) in patients CRF group than control.

Table 1: Distribution of Demographic variables of the control and patients.

Variable	Controls (n = 60)	Patients CRF (n= 60)
Age Mean Rang	 43.183 ± 1.790 (20 – 70)	 42.183±1.790 (20-70)
Gender Male % Femal %	 40 (66.66 %) 20 (33.33 %)	 46 (76.66%) 14 (23.33%)

Smoking status No- smoker % Smoker % Light smokers % Heavy smokers %	 42 (67.74%) 18 (30 %) 9 (50 %) 9 (50 %)	 34 (56.60%) 26 (43.33%) 16 (61.53%) 10 (38.46%)
Urea(mg /dl)	64.199 ±33.100	* 75.062 ± 1.040
Creatine(mg /dl)	0.804 ± 0.022	* 4.073 ± 0.136
Hb (g / dl)	12.325 ± 0.161	* 9.991 ± 0.254
PCV(%)	38.889 ± 0.381	* 31.600 ± 0.660

* Significant difference between CRF and control ($P< 0.05$).

Figure (1) show the yield of *CYP2D6* polymorphisms that analyzed by PCR. Different phenotypes of *CYP2D6* polymorphisms show in figure (2) and (3) by using *Bstn1* enzymes in sample of patients and controls respectively.

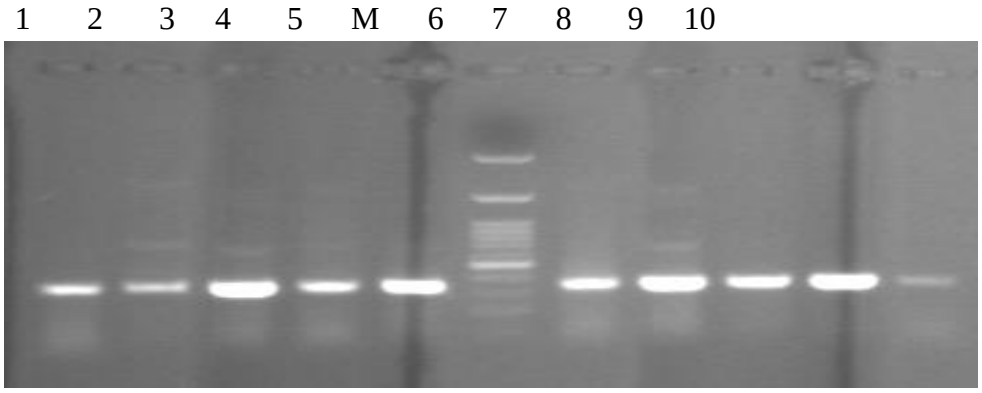

Figure (1) PCR Product For *CYP2D6* Gene.
Lane M Marker
Lane 1-5 Sample Control
Lane 5-10 Sample patients

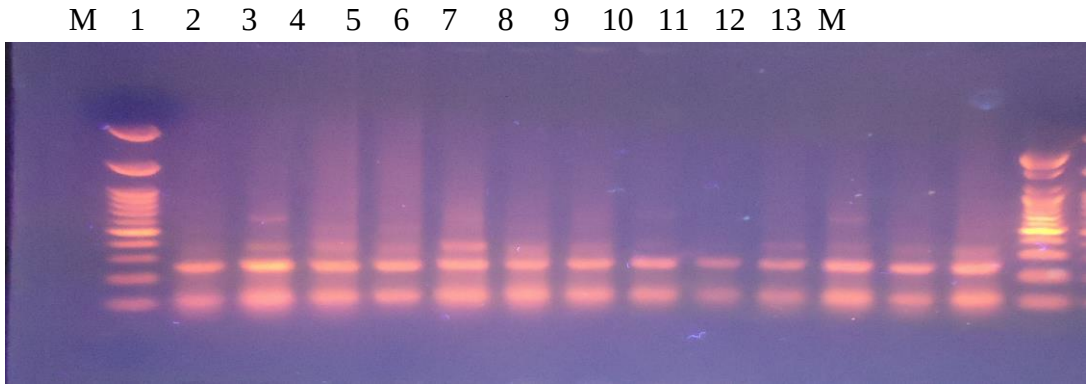

Figure (2) RFLP Analysis Using *BstnI* Restriction Enzyme
*All Samples to the patient .

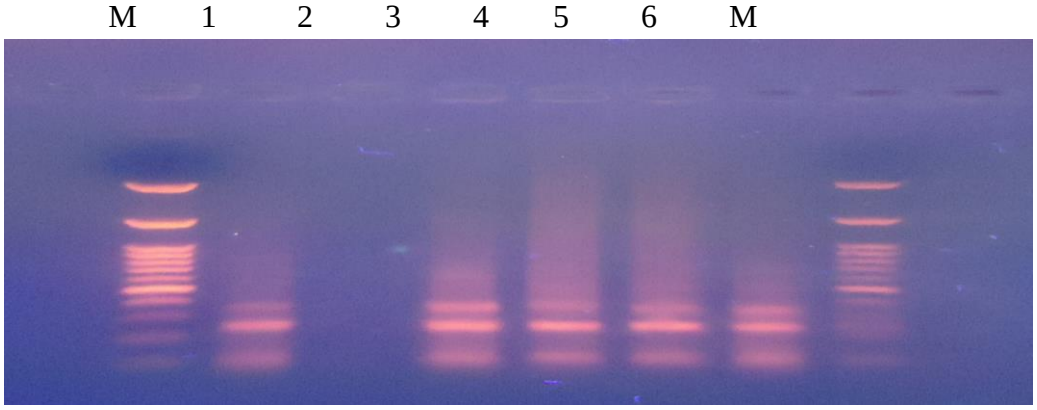

Figure (3) RFLP Analysis Using *BstnI* Restriction Enzyme
*All Samples to the patient .

Genotype distribution of *CYP2D6* alleles among healthy subjects and CRF patients is shown in table (2) in the patient group (65%) (39 of 60) were (EM) and (31%) (19 of 60) were (HEM); (3.33%) (2 of 60) were (PM). In the control group 70% (42 of 60) (EM) and (30%) (18 of 60) were (HEM) . No significant difference between the control group and the patients in (EM) and (HEM) and the increased risk of CRF within OR of (1.13) in contrast there was a (1.8) fold increased risk for this disease with (PM) genotype within OR of (1.18) (95 % CI=) .

Table 2: Distribution of polymorphism of *CYP2D6* gene among patients and controls.

Genotype	Controls (n= 60)	Patients (n=60)	OR	95 CI % I
EM	42(70%)	39(65%)	1.0	1.31-3.8
HEM	18(30%)	19(13.66%)	1.13	0.57-25.0

PM	---------	2(3.33%)	1.89	0.18-12.32

The frequency of the (HEM) genotype according to the age of patients showed an increased risk of CRF in group (41-50) year (OR=0.95 ; 95 % CI = 0.39-4.52). Table (3).

Table 3 : The relationship between *CYP2D6* genotypes and age group in patients.

Age group (years)	NP	EM	HEM	OR	95 % CI	P value
(20 – 30)	10	6(10%)	4(6.66%)	1.00	0.012-2-31	0.081
(31 – 40)	10	7(11.60%)	3(5%)	0.75	0.31-6.32	0.101
(41 - 50)	21	15(25%)	6(10%)	0.95	0.39-4.52	0.078
(51 - 60)	12	8(13.33%)	4(6.66%)	1.25	0.75-5.12	0.096
(61 - 70)	5	3(5%)	2(3.33%)	1.33	0.89-4.85	0.387

* The OR, were not calculated for PM as the number was just two.
In the table (4) we found that *CYP2D6* , HEM genotype has more risk for developing CRF in heavy as compared to light smokers .

Table 4 : OR of developing CRF for *CYP2D6* genotypes stratified by states of smoking.

Smoking states	NS	EM	HEM	OR	95 % CI	P value
No smoking	34(56.600)	24(40%)	10(16.66%)	1.0	0.05-2.87	0.0141
Light smoking	15(25%)	10(16.66%)	5(8.33%)	1.2	0.54-2.54	0.012
Heavy smoking	9(15%)	5(8.33%)	4(6.66%)	1.6	0.76-5.43	0.013

Discussion

The human organism is constantly exposed to harmful exogenous factors (Xenobiotics), including drugs and carcinogens which can induce development of many disease. The enzymes, which metabolize xenobiotics are present in the human organism , run in multidirectional way and exnobiotics can transformed into harmful compounds and become potentially pathogenetic (13).

Patients with CRF represent a unique subgroup with a high exposure to naturally occurring endogenous and exogenous toxicants. The patients accumulate waste products, and it has been suggested they may develop an increased dependence on metabolic enzymes to help prevent or limit damage from toxicants (12) the phase I enzyme cytochrome P450 2D6 (*CYP2D6*) and the phase II isoenzymes are of particular interest because they are subjected to genetic polymorphism which results in absent enzyme function(14) many pharmaceuticals are metabolized by *CYP2D6* (15) .

The mean age of CRF patient was (42.183 ± 1.790) and the maximum number of patients were in the age group of (41-50) years then (51-60) years and this result agree with (16) who found the mean age of men was (54.37 ± 16.9) and women was (49.75 ± 18.09) and the maximum number of patients in the age group of (40-60) years . There is also (17) found the maximum number of age was (45-54) years . a similar trend has also been reported by (18). CRF more frequently in older people and there fore this likely to increase in the population as whole. The reigning study.

(66 %) of the patients were males and the rest were females indicating the predominance of this disease amongst males. This result agree with result (17) . This might reflect a higher exposure of males to the xenobiotic inducing factors , probably at their workplace .

Evolution of others higher risk factors like smoking showed slight correlation of the disease with this factor . A number of studies have associated this factor with pathogenesis of CRF disease (19, 20, 21) In contrast, (22) failed to detect an association between smoking and CRF. In the present study as majority of patients (43.33%) were smokers compared with the control (30%) were smokers.

The levels of creatine and urea were significantly increased ($P<0.05$) in patient group than health control. The result are in agree with different previous research which indicated that the CRF lead to induce severe physiological and biochemical disturbances in patients CRF. Our results agreement with results (23).

The data in present study showed significant increase in urea and creatine in CRF disease .

The Hemoglobin concentration and PCV were significantly lower ($P<0.05$) in patients CRF compared to those of healthy subjects . The present result agree with result (24) lower Hb and PCV levels in CRF patients. The decrease of PCV value in hemodyzed plasma patients to HD indicates the increased destruction of erythrocytes. In uremic patients , however, increased oxidation stress in RBC may result from multifarious factors such as uremic toxin, hemodialysis (25).

Difference among individuals in responses to drugs caused by mutations in *CYP2D6* gene, which is involved in the metabolism of many xenobiotics . The result of the mutation is individual variability of metabolic activity of isoenzymes which is manifested as a total absence, reduction or increase in the enzyme activity (26,27). In the present study we found (1.89) fold increased risk of CRF with (PM) genotype with an OR of (1.89) (95% CI=0.18 – 12.32) . in contrast , there was no significant association were found between the (EM) genotype and CRF . and no significant association between the (HEM) genotype and CRF (OR=1.13, 95%CI=0.57-25.0) and (3.33%) of patients non functional *CYP2D6*. Whereas this result agreement with result (28) mentioned poor metabolize genotype was present in (13) (5.7%) of (228) CRF patients . The patient of (< 61) years were PM *CYP2D6* (11.5) at the mean age (44±1) and the patients of (≥61) years (6.71) were at the mean age (71 ±1) . but in our study the OR , were not calculated for PM as

the number was just two but the maximum number were in the age group (41-50) years that compared with EM of patient in the age group (41-50) years they were (25%).

CYP2D6 an important role in pharmacology. It has been implicated in the metabolism of nicotine in tobacco smoking (29).

CYP2D6 is involved in the metabolism of debrisaquine, the substrates of debrisoquine hydroxylase include aromatic amines and tobacco nitro samines (30)

The risk for developing CRF disease increased in case of heavy smokers with an OR of (1.6) fold as compared to light smokers (OR= 1.2) , but the difference between the two groups was not very high.

To our knowledge the researches about this association are very limited and this is the first study of an association of *CYP2D6* gene with CRF disease in Iraq.

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مرافقة التعدد الوراثي لجين CYP2D6 لمرضى العجز الكلوي في محافظة القادسية/ العراق.

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الخلاصة

يعد الجين *CYP2D6* من جينات الطور الأول المسؤولة عن تشفير الأنزيمات المؤيضة للعقار، إذ تباين الأشخاص في الاستجابة للعلاج نتيجة للطفرة في الجين للـ *CYP2D6* . غالبا ما يعالج مرضى العجز الكلوي بأكثر من علاج واحد . صممت هذه الدراسة للتحري عن توزيع التعدد الوراثي لجين الـ *CYP2D6* في مرضى العجز الكلوي ولمعرفة دور هذا الجين في زيادة خطورة الإصابة بمرض العجز الكلوي . نفذت هذه الدراسة في (60) عينه لمرضى العجز الكلوي و (60) عينه من متبرعين أصحاء. حللت الطرز الوراثية لجين الـ *CYP2D6* بطريقة (RFLP- PCR).

تشير النتائج إلى إن مستوى الكرياتين واليوريا مرتفعة معنويا عند مستوى احتمالية ($P < 0.05$) لمرضى العجز الكلوي مقارنه مع الأصحاء. بينما تركيز Hb و PCV كانت منخفضة معنويا عند مستوى احتمالية ($P < 0.05$) لمرضى CRF مقارنه مع الأشخاص الأصحاء.

لاتوجد مرافقة معنوية بين خطر الإصابة بمرض العجز الكلوي والطراز الوراثي الطافر متباين الزيجة للجين *CYP2D6* . النمط الوراثي متشابه الزيجة الطافر الفاقد للوظيفة يزيد من خطورة الإصابة بمرض العجز الكلوي بمقدار (1.89) مرة 95%) (CI= 0.18-12.32 . أظهرت هذه الدراسة الدور الوقائي لوجود النمط الجيني (EM) *CYP2D6* و للتقليل من شدة المرض.

لم يظهر التحليل الإحصائي مرافقة معنوية بين العمر والطرز الوراثية لجين *CYP2D6* (HEM, EM).

يزداد خطر الإصابة بمرض العجز الكلوي في حالة التدخين الثقيل بمقدار (1.6) مرة مقارنه مع التدخين الخفيف (OR= 1.2).