Study of the Association of CYP2D6*4 Polymorphism with the Susceptibility of HCV- Related Liver Cirrhosis and Liver Cancer.

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Abstract: Background: CYP2D6 is a member of cytochrome P450 enzymes family which is involved in detoxification of a wide range of xenobiotics and drugs. Several genetic polymorphisms had been shown to affect its activity which may results in increased susceptibility to malignant disorders. **Aim**: to detect if there is specific cytochrome CYP2D6*4 genotype associated with hepatocellular carcinoma or hepatic cirrhosis among patients with hepatitis C. **Method:** CYP2D6*4 genotyping was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). This study includes 23 patients with hepatic cirrhosis, 26 patients with HCC and normal 19 subjects with matched age and sex. **Results:** The frequency of (Extensive metabolizers) EM genotype (wild type) was higher in HCC cases compared to cirrhotic patients and controls (76.8% versus 39.1% and 63.2%).The frequency of (intermediate metabolizers) IM genotype (heterozygous variant) was higher in cirrhotic cases compared to HCC patients and controls (52.2% versus 15.4% and 26.3 %). On contrary, the frequency of (poor metabolizers) PM genotypes (homozygous variant) was the lowest among HCC patients in comparison to cirrhotic patients and controls (3.8% vs 8.7% and 10.5% respectively). Higher frequency of IM and PM genotypes were observed in patients more than 45 years old in cirrhotic and malignant patients. Frequency of IM and PM were significantly higher in males than females in HCC patients (p=0.000). Frequency of p allele was higher in males than females and in older patients than younger patients in the three groups. **Conclusions:** These data indicate that PM CYP2D6*4 genotype has no role in development of HCC and IM genotype may have a role in developing hepatic cirrhosis, while higher frequency of EM genotype may contribute to the progression of HCC in HCV-infected subjects

[Sohair K. Sayed and Hala M. K. Imam. **Study of the Association of CYP2D6*4 Polymorphism with the Susceptibility of HCV- Related Liver Cirrhosis and Liver Cancer.** *Life Sci J* 2012;9(3):1571-1577] (ISSN:1097-8135). http://www.lifesciencesite.com. 228

Keywords: CYP2D6*4, genetic polymorphism, hepatitis C virus, cirrhosis, hepatocellular carcinoma

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most prevalent malignant tumor worldwide and ranks the third as a cancer killer, causing more than half million deaths annually [1, 2]. Chronic hepatitis B and C viral infections have been well characterized to play a major role in the HCC etiology. The rates of HCV in Egypt are among the highest in the world, with a prevalence rate of up to 20% [3-5]. However, not all the individuals infected with HBV or HCV develop HCC, other risk factors including environment and genes may be involved in the multistage process of this complex disease [6]. Several studies have reported candidate cytochrome P450 (CYP) genes in which certain alleles are likely to be associated with cancer susceptibility [7].

The cytochrome P450 system is a group of enzymes that are responsible for metabolizing many endogenous and exogenous substances including xenobiotics such as procarcinogens, drugs, and environmental pollutant into more hydrophilic substances [8]. CYPs are encoded by at least 50 different genes grouped in 10 families and sharing approximately 40% sequence homology and are mainly expressed in the liver. In humans, 90% of all of the drugs currently approved for clinical use are metabolized by one of seven CYP isoforms, CYP1A2, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1 and/or CYP3A4 [9,10]. As CYP-mediated bioactivation is an initial and obligatory step in chemical carcinogenesis, polymorphisms associated with altered enzyme activities may influence susceptibility to cancer, and specific combinations of alleles may ultimately provide the genetic fingerprint of one's individual ability to respond to chemical and physical environmental agents, endobiotics compounds or infectious agents [11]. This means that altered levels of *CYPs* might be related to hepatocarcinogenesis. It has also been shown that the genetic polymorphisms of *CYP2E*, *CYP2D6* and *CYP2C19* are associated with the development of HCC [12, 13].

CYP2D6 is perhaps the most extensively studied polymorphically expressed drug metabolizing enzyme in humans and its polymorphism has a high clinical importance [14]. CYP2D6 isoform metabolizes more than 25% of most common drugs [15]. The CYP2D6 gene is localized on chromosome 22q13.1. The locus contains two neighboring pseudogenes, CYP2D7 and CYP2D8. The presence of the highly similar closely

located pseudogenes carrying detrimental mutations have through, for example, unequal crossover reactions led to the formation of many of the variant CYP2D6 alleles, which most commonly encode defective gene products [14] . As a result of the presence of more than 70 allelic variants of CYP2D6 gene [16], metabolism and excretion rates of drugs vary between individuals, from extremely slow to ultra-fast. Different phenotypes can be distinguished: poor metabolizers (PM) lack the functional enzyme; intermediate metabolizers (IM) carry 2 different alleles, leading to partial activity; efficient metabolizers (EM) have 2 normal alleles; efficient intermediate metabolizers (EIM) are heterozygous for 1 deficient allele; and ultra-rapid metabolizers (UM) have multiple gene copies [14] . The most frequent inactivating mutation among Caucasians is the splice site G1934A transition (CYP2D6*4 allele) causes a truncated protein. G to A transition at the intron3/exon4 boundary of the CYP2D6 gene leads to incorrect splicing of mRNA resulting in a frame shift and premature termination [17,18] . This allele was previously called CYP2D6B and accounts for more than 70% of all the inactivating alleles in Caucasian populations [19]. This mutation result in decreased or lack of CYP2D6 isoenzyme activity, leading to PM phenotype [20] , increased risk for adverse side effects or therapeutic failure following drug treatment [21] and deficient hydroxylation of several classes of commonly used drugs, environmental toxic chemicals, and endogenous substances[22].

The aim of this study was to detect if there is specific cytochrome CYP2D6*4 genotype associated with hepatocellular carcinoma or hepatic cirrhosis among patients with hepatitis C.

2. Material and Methods:

Patients:

A total of 49 anti-HCV positive patients recruited from gastroenterology unit of Internal Medicine department, Assiut University, Assiut , Egypt were included in the study; 23 patients with liver cirrhosis and 26 patients with hepatocellular carcinoma. Nineteen healthy volunteers with matched age and sex were included in the study. Liver cirrhosis was diagnosed by clinical manifestation (including coagulopathy, hypoalbuminemia, abnormal liver function tests, haematologic evidence of hypersplenism, ascites, jaundice and portal hypertension with or without variceal bleeding), histological findings of liver biopsy and/or abdominal ultrasonography. The diagnosis of HCC was confirmed by a pathological examination or alfa-fetoprotein (AFP) elevation (>400 ng/ml) combined with positive imaging (Magnetic resonance imaging, MRI and/or computerized tomography, CT) The control subjects had no history of any kind of cancer at the time of ascertainment. All subjects gave their informed consent to participate in this study.

Methods:

Eight ml venous blood was collected and divided into: 3ml in tube containing EDTA (ethylene diamine tetra-acetic acid) for complete blood count and DNA isolation, 1.8 ml in tube containing Na citrate for prothrombin time, concentration and INR and the remaining blood were collected in plain tubes. The samples were centrifuged within 30 minutes at 3000rpm for 10 minutes and the serum samples then collected and divided into aliquots and stored at-70^{o}C for further analysis.

Liver function tests were assayed on BME Hitachi 911 auto-analyzer. Serum AFP was measured using chemo luminescence assay on IMMULIT 1000 and specific assay kits (Siemens Lot. No LAPI 0060). Serum HBV markers, antibodies to hepatitis C virus were detected by commercially available micro particle enzyme immunoassay kits (AXSYM, Abbott Laboratories, Germany).

Cytochrome P450-2D6*4 genotyping:

Genotyping of CYP2D6*4 gene was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technique according to the method described by **Lee *et al.[23]*** DNA isolation was performed by QIAamp DNA mini kit. Cytochrome P450-2D6*4 gene detection was performed by PCR using a set of primer sequences which was designed by **Schur *et al.*** [24] containing the site of the polymorphism (G-to-A transition at position 1934A) as follow: the forward primer 5'-GCCTTCGCCAACCACTCCG-3' and the reverse primer: -5'-AAATCCTGCTCTTCCGAGGC-3'. A total 25 µl reaction volume for PCR amplification included, 12.5µl of 2xGo Taq Green PCR Master Mix (Promega Corporation, US), 2µl of each forward and reverse primers, 5µl template DNA and completed with nuclease- free water to 25µl. The amplification profile was as follow (denaturation at 95ºC for 4 minutes, 30 cycles of: 94°C for 1 minute then ,60ºC for 1 minute then 72ºC for 1.5 minutes and final 72ºC for10 minutes). In parallel with the samples, negative controls containing no DNA were run .The product of PCR amplification were subjected to agarose gel electrophoresis using 1.8 % agarose gel containing ethedium bromide for one hour at 100 volts (355 base pair PCR fragment was indicative of the presence of the gene).

Digestion of the amplified product by Mva-1 **restriction enzyme:** 10 µl of the PCR amplified product was incubated with 0.2µl (2 units) of the restriction endonuclease Mva-1 enzyme, 2.0µl of appropriate buffer (50 mM tris HCL, 100m M NaCl, 10 mM EDTA, 1mM dithiothreitol, pH 7.5 at 37ºC) and 7.8 µl of DNA/RNA free water at 37 ^{o}C for 4 hours. The digested products were electrophoresed on

2% Agarose gel containing Ethidium Bromide. Then the gel was visualized on an UV light for genotyping. Normal allele of CYP2D6*4 (EM) produces two fragments of 250 and 105 bp after digestion with Mva-1 restriction enzyme. G to A transition at position 1934 abolishes the restriction site and a fragment of 355 bp is observed. Heterozygous individuals (IM) show normal allele (250, 105 bp) and one mutated allele of 355 bp and homozygous individuals (PM) showing 355 bp band.

Statistical analysis:

Data were statistically described in terms of range; mean ±standard deviation ±SD, frequencies (number of cases) and relative frequencies (percentages) when appropriate. SPSS Version 17.0 (SPSS Inc., Chicago, IL) was used for all data analyses. Chi-square or Chi Square test were used for contrasts involving categorical variables. For comparisons involving continuous variables, the two-sided two-sample Student *t* test, Welch Modified *ANOVA* were used in cases where the normal distribution assumption or equal variance assumptions were not viable. Odd Ratio (OR with its 95% CI was calculated for the occurrence of PM and IM between cases and controls) A probability value (*p* value) less than 0.05 was considered statistically significant.

3. Results:

Patient's characteristics are summarized in table (1). In table (2) *CYP2D6*4* genotyping data were combined to express 3 categories, as previously described: extensive (EM), intermediate (IM) and poor metabolizers (PM) , frequencies of 3 CYP2D6*4 categories among healthy subjects were 63.2% for EM, 26.3% for IM and 10.5% for PM, in agreement with published data, **Vineis *et al.*** [25]. Genotype distribution differed among the two patients groups; in particular, PM was reduced among HCC compared with cirrhosis patients (3.8 % *vs.* 8.7 %), however there was no statistical significant difference. This finding suggests that PM genotypes may behave as a protection factor in severe liver lesions. On the other hand, EM was significantly increased in HCC group in comparison to cirrhotic group (p=<0.01). As an estimate for the relative risk, the odds ratio for EM genotype was 6.533 with a 95% confidence interval of 1.807- 23.627. These data indicate a higher risk for HCC in individuals carrying the EM CYP2D6*4.

When data was compared with respect to age of the patients, there was higher frequency of IM and PM observed in patients more than 45 years old in cirrhotic and malignant patients. However, frequency of EM was higher in patients less than 45 years old in cirrhotic, malignant and control groups. Frequency of p allele was higher in the three groups in individuals more than 45 years old (Table 3).

When data was compared with respect to sex of the patients, there was higher frequency of IM and PM observed in males compared to females in cirrhotic and HCC group; however there was no statistical significant difference. Frequency of p allele was higher in males than females in the three groups (Table 4).

Table (1): Demographic and Clinical Data in cirrhotic, malignant and control groups

Item	Cirrhotic group N(23)	Malignant group (26)	Control N(19)	*p-value*
Age **Mean ±SD**	49.23±15.01	55.19±8.68	56.84±7.84	N.S
Sex **Male** **Female**	18 (78.3%) 5(21.7%)	22(84.61%) 4(15.4%)	11(57.91%) 8(42.1%)	N.S
Hepatomegaly + -	3(17.61%) 14 (82.4%)	10(38.5%) 16(66.5%)	--	<0.01
Splenomegaly + -	14 (82.4%) 3(17.61%)	13(50%) 13(50%)	--	<0.000
Jaundice + -	7(41.2%) 10(58.81)	10(38.5%) 16(66.5%)	--	<0.001
Ascites + -	9(52.9%) 8(47.1%)	11(42.3%) 15(57.7%)	-	<0.001

p<0.05 is significant

Table (2): Frequency of Cytochrome CYP2D6*4 Genotype in cirrhotic, malignant and control groups:

Item	Cirrhotic group N(23)	Malignant group N(26)	Control N(19)	*p-value*
PM	2(8.7%)	1(3.8%)	2(10.5%)	0.453
IM	12(52.2%)	4(15.4%)*	5(26.3%)	<0.000
EM	9 (39.1%)	21(76.8%)*	12(63.2%)	<0.01

EM= Extensive metabolizer, IM= Intermediate metabolizer, PM= Poor metabolizer, , * *p*-value <0.05 , * Malignant vs. Cirrhotic

Table (3): Association of CYP2D6*4 genotypes and allelic frequency with age :

	EM (No, %)	IM (No, %)	PM (No, %)	E allele frequency	P allele Frequency
Hepatic cirrhosis					
<45 (no=5)	3 (60.0%)	2 (40.0%)	0 (0.0%)		
≥45 (no=18)	6 (33.3%)	10 (55.6%)	2 (11.1%)	0.80	0.20
P value	0.283	0.455	0.605	0.61	0.39
Malignant liver					
<45 (no=1)	1 (100%)	0 (0.0%)	0 (0.0%)	1.00	0.0
≥45 (no=25)	20 (80%)	4 (16%)	1 (4%)	0.88	0.12
P value	0.808	0.846	0.962		
Controls					
<45 (n=8)	7 (87.5%)	1 (12.5%)	0 (0.0%)	0.93	0.07
≥45 (n=11)	5 (45.4%)	4 (36.4%)	2(18.2%)	0.63	0.37
P value	0.244	0.366	0.386		

EM= Extensive metabolizer, IM= Intermediate metabolizer, PM= Poor metabolizer, E= Extensively metabolizing allele, p<0.05 is significant

Table (4): Association of CYP2D6*4 genotypes and allelic frequency with sex :

	EM (No, %)	**IM (No, %)**	**PM (No, %)**	**E allele frequency**	**P allele frequency**
Hepatic cirrhosis					
Male (no= 18)	6 (33.3%)	10 (55.5 %)	2(11.1%)	0.61	0.39
Female (no=5)	3 (60%)	2 (40%)	0 (0.0%)	0.80	0.20
***P* value**	0.283	0.455	0.605		
Malignant liver					
Male (no=22)	17(77.3%)	4 (18.1%)	1 (4.5%)	0.86	0.14
Female(no= 4)	4 (100%)	0 (0.0%)	0 (0.0%)	1.0	0.0
***P* value**	0.400	0.489	0.846		
Controls					
Male (n=11)	5 (45.5%)	4 (36.4%)	2(18.1%)	0.63	0.37
Female (n=8)	7(87.5%)	1(12.5%)	0 (0.0%)	0.93	0.07
***P* value**	0.080	0.267	0.322		

EM= Extensive metabolizer, IM= Intermediate metabolizer, PM= Poor metabolizer, E= Extensively metabolizing allele , ***P*** <0.05 is significant

4. Discussion:

Carcinogenic process in patients with HCV-associated liver disease, mainly cirrhosis, is thought to involve multiple steps, and many risk factors have been proposed. In the clinical setting some patients with HCV-associated cirrhosis progress to HCC, while others never develop HCC. Such a difference in progression to HCC is clinically important, and the factor(s) affecting this difference should be elucidated in such patients. However, this issue remains unsolved. One of the many risk factors is the patient's capability to metabolize xenobiotics (including drug), because some xenobiotics play a role in inducing cancer as procarcinogen or carcinogen [7].

In the present study, Cirrhotic patients, HCC patients and healthy control subjects were compared with respect to genotype frequencies of CYP2D6*4, which have been suggested to alter the enzyme activity. The frequency of mutation of the CYP2D6 allele (PM) was lower in HCC patients than in cirrhotic patients or healthy subjects, however, no statistical significant difference was found. This finding was in agreement with the study of **Agúndez *et al.*** [26], who used the RFLP method and reported that the frequency of mutation of the CYP2D6 allele (PM) is lower in HCC patients than in cirrhotic patients or healthy subjects. They found no significant difference in the frequency of the CYP2D6 allele between the HCC patients and healthy controls. Also, **Kimura *et al.*** [27] found no significant differences between HCC patients and controls in the frequency of the allele. Another study was conducted by **Mochizuki *et al.*** [7] who compared the frequencies of mutation of CYP2D6 allele in healthy subjects with HCC patients. They extracted the genetic polymorphic frequencies of CYP subtypes in healthy Japanese subjects from the review of **Shimizu *et al.*** [28] that summarized the data from many reports and compared the frequencies of mutant alleles in healthy subjects with HCC patients; they found no statistically significant differences in mutant alleles between the two groups. On the contrary to our findings, **Silvestri *et al.*** [11] found an association between the CYP2D6 polymorphism and cirrhosis in HCV patients and hepatomas in patients with chronic liver diseases. They examined the prevalence of various genotypes of CYP2D6 in four groups of patients with HCV: asymptomatic carriers, patients with active hepatitis, cirrhotic patients and patients with hepatoma.

CYP2D6 genotypes have an effect on liver disease progression as shown by the distribution of different genotypes according to the severity of liver lesions [11]. Several studies have shown that the extensive metabolizer (EM) phenotype is associated with increased risk of various cancers [29]]. The relationship of *CYP2D6* genotypes with liver cancer has been explored by **Agundez *et al.*** [26] in a case-control study that compared hospital controls and HCC patients with different etiologies. A significant association with risk was observed for high activity alleles. Our data confirmed these observations as the frequency of EM phenotype was significantly higher in HCC group than in cirrhotic group and normal control (76.8 % vs 39.1 and 63.2% %).The over-representation of CYP2D6*4 EM among HCC patients can be explained in two ways: first, the CYP2D6*4 may mediate the activation of procarcinogenic agents present in environment. Second, the CYP2D6*4 gene may be in a linkage disequilibrium with the causative gene.

It is noteworthy that patients carrying IM or PM genotype with less metabolic activity may not be able to respond to the treatment. Moreover, these patients when treated with normal amount of drug dose, the drug will accumulate in the body and ultimately turns into carcinogen. Alternately, if these patients were treated with lower drug doses, it might lead to drug resistance and poor prognosis. Hence these patients must be treated with caution [30]. In this study, IM genotype frequency was statistically significant higher in cirrhotic group than malignant group (52.2% vs 15.4%). These facts suggested a role for IM CYP2D6*4 in hepatic disease severity and its deterioration toward cirrhosis.

Ageing is the progressive accumulation of more or less random changes that lead to time-related loss of functional units e.g. nephrons, alveoli, or neurons. These time-related changes may explain partly the increased inter-individual variability occurring in drug disposition as people get older [31]. Investigations on the influence of ageing on phase I enzymes in humans have reported conflicting results. Indeed, a study conducted in 54 liver samples from healthy donors from 9 to 89 years did not show changes in microsomal protein content, total P450 or NADPH cytochrome P450 reductase activity with age. By contrast, another study carried out in 226 subjects with histopathologic changes of the liver revealed a significant decrease of 32% in total cytochrome P450 content of liver biopsy samples in subjects >70 years as compared to young adults [32].

In the current study, increased frequency of IM (55.6% and 16%) and PM (11.1% and 4%) were observed in cirrhotic patients and HCC patients more than 45 years old respectively .In contrary to our findings, **Sailaja *et al.*** [33] demonstrated that, patients less than 20 years of age have increased frequency of IM (28.57%) and PM (4.76%) genotype with corresponding increase in P allele frequency. This difference in our results can be referred to the age groups of our patients as most of them were higher than 45 years old and nobody less than 20 years old. Also, the differences in the results obtained in previous researches can be explained by the inter-

individual variability in xenobiotic metabolism and frequencies of these polymorphisms are variable in different populations due to different ethnic backgrounds, environment and lifestyle**.**

In the current study, when data was compared with respect to sex of the patients, there was higher frequency of IM (55.5% and 18.1%) and PM (11.1% and 4.5%) observed in males compared to females in cirrhotic and HCC patients, with higher p allele frequency in males. The present study was in agreement with the study of the **Sailaja *et al.*** [33] who stated that P allele frequency was increased in male patients and PM genotype was found only among male patients.

Conclusion:

PM CYP2D6*4 genotype has no role in development of HCC but may behave as a protection factor int severe liver lesion. IM genotype may have a role in developing hepatic cirrhosis. The high activity allele (EM genotype) associated with HCC may contribute to the progression of HCC in HCV-infected subjects. CYP2D6*4 might serve as a genetic non-invasive marker, enabling identification of HCV-infected patients that are prone to develop cirrhosis or HCC early in the course of the infection.

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References:

1. Bosch FX, Ribes J, Diaz M, Cleries R. (2004): Primary liver cancer: worldwide incidence and trends. Gastroenterology; 127(5 Suppl 1):S5–S16.
2. Parkin DM, Bray F, Ferlay J, Pisani P. (2005): Global cancer statistics, 2002. CA Cancer J Clin ;55(2):74–108.
3. Wasley A, Alter MJ. (2000): Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* ; 20: 1–16.
4. Arafa N, El Hoseiny M, Rekacewicz C *et al.* (2005): Changing pattern of hepatitis C virus spread in rural areas of Egypt. *J Hepatol* ; 43: 418–24.
5. Lehman EM., Soliman AS., Ismail K. *et al.* (2008): Patterns of hepatocellular carcinoma incidence in Egypt from a population-based cancer registry. Hepatology Research ; 38: 465–473.
6. Li R, Shugart YY, Zhou W, *et al.* (2009): Common genetic variations of the cytochrome P450 1A1 gene and risk of hepatocellular carcinoma in a Chinese population. Eur J Cancer. ; 45(7):1239- 1247.
7. Mochizuki J, Murakami S, Sanjo A, Takagi I, Akizuki S, Ohnishi A.(2005): Genetic polymorphisms of cytochrome P450 in patients with hepatitis C virus-associated hepatocellular carcinoma. J Gastroenterol Hepatol. ; 20(8):1191-1197.
8. Rogers JF, Nafziger AN and Bertino JS Jr. (2002): Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. Am J Med ; 113: 746-750.
9. Guengerich F P. (2001): Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. Chemical Research in Toxicology ; 14(6): 611-650.
10. Nebert DW and Russell DW. (2002): Clinical importance of the cytochromes P450. Lancet; 360(9340):1155-1162.
11. Silvestri L, Sonzogni L, De Silvestri A *et al.* (2003): CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer. Int J Cancer ; *104*: 310-317.
12. Chau TK, Marakami S, Kawai B, Nasu K, Kubota T and Ohnishi A (2000): Genotype analysis of the CYP2C19 gene in HCV seropositive patients with cirrhosis and hepatocellular carcinoma. Life Sci ; *67*: 1719-1724.
13. Iizuka N, Oka M, Hamamoto Y, *et al.* (2004) : Altered Levels of Cytochrome P450 Genes in Hepatitis B or C Virus-infected Liver Identified by Oligonucleotide Microarray. Cancer Genomic & Proteomics ; 1: 53-58.
14. Ingelman-Sundberg M. (2005): Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J.; 5:6-13.
15. Burroughs VJ, Maxey RW, Levy RA. (2002) :Racial and ethnic differences in response to medicines: toward individualized pharmaceutical treatment. J. Natl. Med. Assoc. 94:1-26.
16. Scordo MA, Caputi AP, D'Arrigo C, Fava G, Spina E. (2004): Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. Pharmacol. Res.; 50:195-200.
17. Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, Wolf CR. (1990): Identification of the primary gene defect at the cytochrome P450 CYP2D locus. Nature. 25; 347(6295):773- 776.
18. Surekha D, Sailaja K, Rao DN, Padma T, Raghunadharao D, Vishnupriya(2010):

Association of a CYP17 gene polymorphism with development of breast cancer in India. Asian Pac J Cancer Prev. ;11(6):1653-7.
19. Hirvonen A. (1999): Polymorphisms of xenobiotic-metabolizing enzymes and susceptibility to cancer. Environ Health Perspect. ; 107 Suppl 1:37-47.
20. Van Der Weide J, Steijns L. (1999): Cytochrome P450 enzyme system: genetic polymorphisms and impact on clinical pharmacology. *Ann. Clin. Biochem*. 36:722-9.
21. Lavandera JV, Parera VE, Batlle A, Buzaleh AM.(2006): CYP2D6 polymorphisms in patients with porphyrias. Mol Med. ;12(9-10):259-63.
22. Stamer UM, Bayerer B, Wolf S, Hoeft A, Stüber F (2002): Rapid and reliable method for cytochrome P450 2D6 genotyping. Clin Chem.; 48(9):1412-7.
23. Lee HC, Yoon YB, Kim CY.(1997):Association between genetic polymorphisms of the cytochromes P-450 (1A1, 2D6, and 2E1) and the susceptibility to pancreatic cancer. Korean J Intern Med. ;12(2):128-36.
24. Schur,B.C.; Bjereke,J.; Nuwayahid,N. And Wong,S.H.(2001): "Genotyping of cytochrome P450-2D6*4 and *3 mutation using conventional PCR". Clin.Chem.Acta., 308:25-31.
25. Vineis P, Malats N, Lang M, d'Errico A, Caporaso N, Cuzick J, Boffetta P.(1999): Metabolic polymorphisms and susceptibility to cancer. IARC Scientific Publications. Vol. 148. Lyon: International Agency for Research on Cancer.
26. Agundez JA, Ledesma MC, Benitez J, Ladero JM, Rodriguez-Lescure A, Diaz-Rubio E, Diaz-Rubio M. (1995): CYP2D6 genes and risk of liver cancer. Lancet ;345:830 –1.
27. Kimura Y, Selmi C, Leung PS, *et al.* (2005): Genetic polymorphisms influencing xenobiotic metabolism and transport in patients with primary biliary cirrhosis. Hepatology. ; 41(1):55-63.
28. Shimizu T, Ochiai H, Åsell F *et al.* (2003): Bioinformatics research on inter-racial difference in drug metabolism. I. Analysis on frequencies of mutant alleles and poor metabolizers on CYP2D6 and CYP2C19. Eur. J. Drug Metab. Pharmacokinet. ; 18: 48–70.
29. Raunio H, Husgafvel-Pursiainen K, Anttila S, Hietanen E, Hirvonen A, Pelkonen O.(1995):Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility--A Review. Gene. 14; 159(1):113-21.
30. *Hussein* S*, Abd El-Aal*l A*, Afify* R *and Noha El-Husseiny* N (2010): study of the *association of* CYP2D6*4 polmorphism with the risk of chronic myeloid leukemia .Egypt J. Med. Lab. Sci. ; 19(2):71-78.
31. Mangoni AA, Jackson SHD (2004): Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. Br J Clin Pharmacol.; 57: 6-14.
32. Wauthier V, Verbeeck RK and Buc Calderon P(2007): The Effect of Ageing on Cytochrome P450 Enzymes: Consequences for Drug Biotransformation in the Elderly *Current Medicinal Chemistry;* 745: 757- 745.
33. Sailaja K, Vishnupriya S, Surekha D, Nageswara Rao D, Raghunadha Rao D (2007): Association of CYP2D6*4 polymorphism with chronic myeloid leukemia. J. of Med Science Res, 1(1).

7/22/2012