Study the Cytochrome P450 Gene Expression Changes in Iraqi Patients with Chronic Liver Disease

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Abstract

The cytochrome P450 is a chemical group of heme-containing proteins speaks to one of the biggest and most practically various superfamilies' found in nature. Chronic liver disease (CLD) is a the process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. The aim of this study to assessment the cytochrome P450 gene expression changes of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease(hepatitis+ alcoholism and hepatitis+ non-alcoholism).CYP1A2, CYP2B6, and CYP2E1 mRNA gene expression were assessed by quantitative real time PCR (qRT-PCR) in 50 cases with CLD and 50 subjects as control. Primers for genes of interest, CYP1A2, CYP2B6, and CYP2E1mRNA and housekeeping gene (GAPDH) were designed by using NCBI tools. Fatty acid synthase (FAS) was estimated by ELISA technique. The results showing statically significant differences in FAS levels (ng/ml) between study groups, HC, HNC, and control (p-value< 0.05). GEF in CYP1A2 gene showing no change between HC and control but there were statistical variations in GEF in HNC and both HC or control. GEF in CYP2B6 and CYP2E1 genes showing highly significant differences between control and both HNC and HC. In conclusion, CYP2B6 and CYP2E1 gene expression were risk factor for progression HC but not HNC through stimulation of increasing levels of FAS.

***Keywords**: chronic liver disease, cytochrome P450, Fatty acid synthase, gene expression.*

Introduction

The cytochrome (P450) is a chemical group of heme-containing proteins speaks to one of the biggest and most practically various superfamilies' found in nature [(1)].The primary capacity of P450s is to encourage the biotransformation of mixes by expansion of practical gatherings reasonable for conjugation and extreme disposal from the life form. The aim of this study to assessment the cytochrome P450 gene expression changes in patients with chronic liver disease [(2)]. In the clinical context, Chronic liver disease (CLD) is a the process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis [(3)]. Patients with either diagnosed or undiagnosed chronic liver disease occasionally present with an acute deterioration of liver function caused by direct or indirect insults to the liver [(4)]. The expression of CYP 450 enzymes is influenced by endogenous factors, such as genetic polymorphisms, gender, age, and the levels of endocrine hormones [(5)].The expression of CYP45 enzymes is also influenced by exogenous factors such as drugs and environmental chemicals, as well as the physic-pathological conditions [(6)]. CYP1A2 is a member of the cytochrome P450 with a mixed-function of oxidase system, it is involve in the metabolism of xenobiotics in the body and in humans, the CYP1A2 enzyme is encoded by the CYP1A2 gene [(7)]. This quality, CYP2B6, encodes an individual from the cytochrome P450 superfamily of proteins. The cytochrome P450 proteins are mono-oxygenates which catalyze numerous responses engaged with medication digestion and amalgamation

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of cholesterol, steroids and different lipids. This protein restricts to the endoplasmic reticulum and its appearance is initiated by phenobarbital. The catalyst is known to use some xenobiotic, for example, the counter malignant growth drugs cyclophosphamide and ifosphamide [8]. CYP2E1 is a layer protein communicated in significant levels in the liver, where it makes about half out of the absolute hepatic cytochrome P450 mRNA[7] and 7% of the hepatic cytochrome P450 protein.[8] The liver is thusly where most medications experience deactivation by CYP2E1, either legitimately or by encouraged discharge from the body.CYP2E1 uses for the most part little, polar particles, including dangerous research facility synthetic substances, for example, dimethyl formamide, aniline, and halogenated hydrocarbons. While these oxidations are frequently of advantage to the body, certain cancer-causing agents and poisons are bio activated by CYP2E1, involving the catalyst in the beginning of hepatotoxicity brought about by specific classes of medications [9]. CYP2E1 also carries out the metabolism of endogenous fatty acids such as the ω-1 hydroxylation of fatty acids such as arachidonic acid, involving it in important signaling pathways that may link it to diabetes and obesity. Thus, it acts as a mono-oxygenase to metabolize arachidonic acid to 19-hydroxyeicosatetraenoic acid (19-HETE) [10]. The aim of this study to assessment the cytochrome P450 gene expression changes of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease (CLD), (hepatitis +alcoholism and hepatitis +non-alcoholism).

Materials and Method

1- Subjects of the study:

The samples of fresh blood were collected in GIT Centre in Merjan teaching hospital from 50 patients with CLD and 50 healthy subjects as control group. The aged and gender of both groups were matched (p-value> 0.05).

2-Methods: Gene Expression Analysis of CYP1A2, CYP2B6, and CYP2E1

Total RNA was extracted from fresh blood of patents and control by using the TRIzol reagent (USA). The concentration of total RNA was measured by spectrophotometry and the OD260/OD280 ratio was obtained to assess the RNA purity. cDNA synthesis performed by reserve transcript and conducted by PrimeScriptTM RT-PCT reagent Kit (Korea) in a 50 µL reaction mixture following the supplier's instructions. The qRT-PCR was performed by using cDNA as a template in the Exicyclere Real-Time PCR System (Bioneer, Korea) with SYBR green kit as fluorescent dye according to the protocol of manufacture. The PCR conditions were 95 □ for 1min, followed by 40 cycles of 95 □ 15 s, 62 □ 45 s. The primers used in the RT-PCR were designed by NCBI tool and not showing here. The relative gene expression levels were calculated on the basis of $2^{-\Delta\Delta Ct}$. GAPDH was conceder as housekeeping gene as control. The results are presented as fold change of CYPs in patients group compare to control (GEF).

3- FAS levels estimation: Assessed by ELISA depending on Elabscince™ protocol by manufacturer instructions provided with kit.

4-Statistical analysis: Data have been analyzed statistically using SPSS program version 21. Analysis of quantitative data was done using t-test and ANOVA analysis.

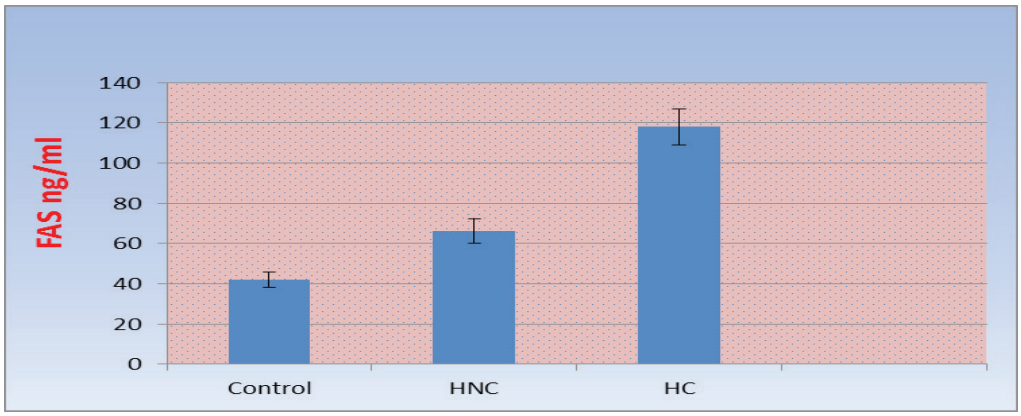
Results

Clinic-pathological characteristics of patients with CLD included in this study are illustrate in table 1:

Table (1): Clinic-pathological characteristics of patients included in this study

Clinic-pathological variables	NO.	%
Total No. of patients	**50**	**100%**
* Age - <30 - ≥30	 22 28	 44 54
* Sex - Male - Female	 26 24	 52 48
*Hepatitis -Hepatitis +Alcoholism (HC) -Hepatitis +Non-Alcoholism (HNC)	 20 30	 40 60

Figure 1, showing the mean±sd of levels of FAS (ng/ml) in patients study group compare to control:



Figure(1): Mean±sd of FAS (ng/ml) in patients groups compare to control

From above figure, there were statically significant differences in FAS levels (ng/ml) between study groups, HC, HNC, and control (p-value<0.05).

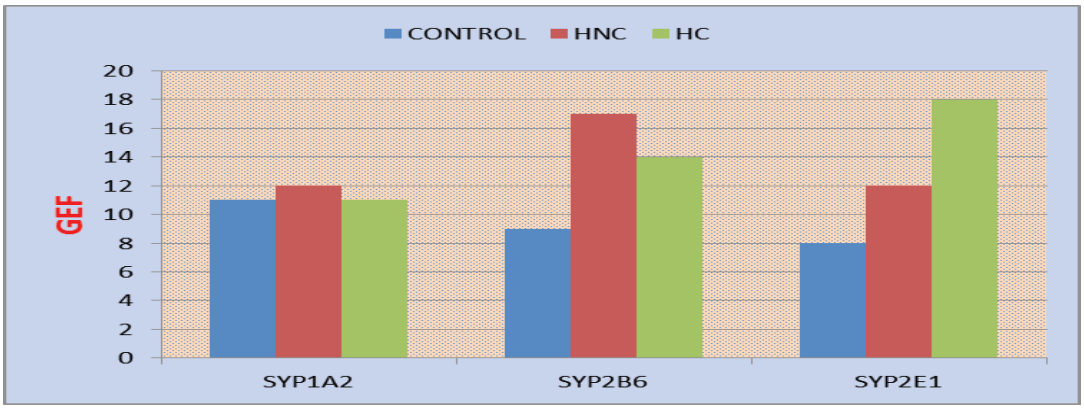
Table 2, showing the levels of FAS (ng/ml) in patients study group depending on age and gender compare to control.

Table (2): **Mean values of FAS levels (ng/ml) for patients compared with control group .**

Clinic-pathological variables	**FAS (mean± SD) ng/ml Age**			**FAS (mean± SD) ng/ml Gender**		
CLD (Hepatitis) -Hepatitis +Alcoholism (HC) -Hepatitis +Non- Alcoholism (HNC)	≥ 30	< 30	p-value	**Male**	**Female**	**p-value**
	123.7±11 67.9±3	113±12 62.3±3	**0.005** **0.048**	123.5±13 68.5±2	122±12 66±1.5	0.084 0.023

From the above table, there were statistical significant differences in levels of FAS ng/ml in HC and HNC subgroups ≥ 30 and < 30 (p-value< 0.05) and this mean that age was risk factor for HC and HNC but the gender in HC was no statistical differences (p-value>0.05) while in HNC the gender was risk factor and increased in males compare to females.

The cytochrome P450 gene expression changes fold of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease (CLD), (hepatitis +alcoholism and hepatitis +non-alcoholism) compare to control group were illustrated in figure 2:



Figure(2): gene expression fold GEF of CYP1A2, CYP2B6, and CYP2E1 genes in study groups

Discussion

The present study examined the fold of major drug metabolizing P450 (CYP1A2, CYP2B6, and CYP2E1) gene expression fold GEF and FAS levels in 50 Iraqi chronic liver disease (CLD) (hepatitis +alcoholism and hepatitis +non-alcoholism). and compared with 50 normal subjects. FAS is a multi-enzyme protein that catalyzes synthesis of fatty acid . FAS is not a single enzyme but a whole enzymatic system composed of two identical 272 kDa multifunctional polypeptides, in which substrates are handed from one functional domain to the next (11). In the present study, the serum FAS levels of the HC patients were found to be significantly higher in comparison to the HNC and healthy controls, indicating that high concentrations of FAS in serum may result from enzyme secretion by abnormal liver cells. Dorn C et al (2010) were reported that the transcriptional induction of FAS expression in hepatic steatosis is impaired in nonalcoholic steatohepatitis, while hepatic inflammation in the absence of steatosis does not affect FAS expression, suggesting that FAS may be serve as a new diagnostic marker or therapeutic target for the progression of nonalcoholic fatty liver disease (12). Li M et al (2018) were suggested that the accumulation of free fatty acids in hepatocytes induces lipotoxicity, leading to non-alcoholic fatty liver disease (13). GEF in CYP1A2 gene showing no change between HC and control and there were no statistical variations in GEF in HNC and both HC or control, this means that CYP1A2 was not involved in progression of HC and HNC to promoting of chronic liver disease. CYP2B6, and CYP2E1 genes were found help to progression of HC and HNC of chronic liver disease because of highly alteration in GEF in different these groups and this agreement with other studies that using genetic markers as diagnostic agents(14,15). These results suggest a significant role of CYP2B6, and CYP2E1 genes in the regulation of hepatic lipid metabolism via the fatty acid synthesis pathway and FAS, a critical factor for lipid synthesis. In conclusion, The identification of effective gene expression of CYP2B6, and CYP2E1 genes and FAS as molecular and bio markers of HC and HNC could improve the early detection of CLD.

Conflicts of Interest: No conflicts of interest to declare in relation to this work.

Acknowledgment: The authors thanks all the staff of GIT Centre in Merjan teaching hospital for contribution.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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