Biochemistry Letters, 13(19) 2018, pages (235-245)

Scientific Research & Studies Center-Faculty of Science- Zagazig University-Egypt

Biochemistry Letters

Journal home page:

Polymorphisms of glutathione peroxidase-1 gene in Egyptian Patients with Chronic Hepatitis C Virus

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ARTICLE INFO

HCV, HCC, GPX1

SNP, Polymorphism

ABSTRACT

Background: Glutathione peroxidase 1 gene (GPX1) is the most common isoform of GPX family .It remove the reactive oxygen species in a continuous process. Since the identification of a well-characterized functional polymorphism named Pro198Leu (C>T) in GPX1 gene, abundant studies have evaluated the association between Pro198Leu polymorphism and tumor risk in diverse population. The present study was planned to evaluate the presence of GPX1 (Pro198Leu) polymorphisms in Egyptian patients with chronic hepatitis C virus.

Methods: Genomic DNA from peripheral blood leukocytes of 243 patients with chronic hepatitis C, 134 of whom were diagnosed as hepatocellular carcinoma (HCC)and 112 healthy controls were enrolledandgenotypedGPX1Pro198Leu polymorphism by polymerase chain reaction_restriction fragment length polymorphism (PCR-RFLP) method. **Results:** A significant difference in the frequencies of Pro/Pro (32.8%) , Pro/Leu (59.7%) and Leu/Leu (7.5%) genotypes in cancer cases and in controls (57.1%,42.9%,0% respectively) were found. The results also indicated that the distribution of the all genotypes (PP,PL,LL) were significantly different between the control and HCV group (P =0.000, 0.01,0.004 respectively).**Conclusion**: The results of this study suggested that GPX1 Pro198Leu polymorphism could be a risk factor for HCV,HCC Egyptian patients. However, largescale studies on the effect of this polymorphism are needed for conclusive understanding.

INTRODUCTION

Chronic hepatitis C virus(HCV) infection is a major risk factor for the development of hepatocellular carcinoma(HCC).It is the fifth most common malignancy and the third leading cause of cancer death worldwide (1).Egypt has one of the highest prevalence rates of HCV infection and HCC is the second most common cancer in men and the the 6th most common cancers in women

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(2).Disease progression is influenced by additional factors such as duration of infection, age at infection, gender, coinfection with HBV, and the level of HCV viramia and its genotype (3).

Oxidative stress is an imbalance between production and elimination of reactive metabolites of oxygen and nitrogen, in favor of their production leading to potential damage. During oxidative stress, biologically important molecules and cells can be damaged, and this can be significant in the pathogenesis of many diseases(4,5).Reactive oxygen species (ROS) is known to activate the apoptosis of some hepatocytes and therefore contribute to inflammation, regeneration, fibrogenesis, and carcinogenesis. The enzyme generally considered to be the frontline defense against ROS is glutathione peroxidase (GPX).It is an important antioxidant selenium-dependent enzyme that catalyses the breakdown of hydrogen peroxide and organic hydroperoxides , resulting in the oxidation of glutathione(GSH) to glutathione disulphide (GSSG) (6).

GPX1 has been reported to be implicated in oncogenesis and progression of several cancer types (7,8)., it's overexpression suppresses intracellular ROS which attenuates growth factor receptor activation mediated by oxidative stress, resulting in decreased cellular proliferation(9).GPX1 is located on chromosome position 3p21 and contains a genetic polymorphism (rs1050450) that results in either a proline (Pro) or leucine (Leu) at codon 198, described to be a risk factor for the development of various cancers, including lung cancer (10) prostate cancer, (11).and bladder cancer (12).

GPX-1 Mills first described GPX activity in 1957 (13). and its function was hypothesized to be protection of red blood cells against hemolysis by oxidation (14).. Activity levels of the antioxidant enzyme GPX1 is likely affected by functional polymorphisms in the genes encoding them. A polymorphism in the GPX1 gene (Pro198Leu), encoding the isoenzyme GPX1, was reported to have a relation to HCC development (15). The genetic polymorphism of glutathione peroxidase-1 may have a significant effect on the enzyme activity. In particular, polymorphism in GPX1 Pro198Leu (C→T) located in the second exon of gene GPX1 has a high level of heterozygosity; it induces a proline (CCC)–leucine (CTC) substitution. Moreover, it may have an effect on the catalytic enzyme activity, its affinity to the substrate, specificity, structure stability, etc (16). With the use of cell lines, it was shown that Pro198Leu enzyme had lower activity compared to wild type protein. Catalytic gene activity was found to be 5% lower in each additional T copy in patients with this allele (17). Genetic variations in the antioxidant gene coding for the GPX1 enzyme may cause decreased or impaired regulation of their enzymatic activity and alter ROSdetoxification. Therefore, genetic variations among these enzymes that protect the cell against ROS may lead to disease (18). Due to the high interaction

potentiality of ROS with genetic material, polymorphisms in genes coding for antioxidant enzymes may play an important role for inter-individual differences in maintaining the human genome's integrity. Genetic polymorphisms in GPX1 have been implicated in proneness to cancer and other diseases (19,20).The present study aimed to evaluate the presence ofGPX1 (Pro198Leu) polymorphisms in Egyptian patients with chronic hepatitis C virus.

2. Patients and methods

2.1. Study population

The current study was carried out on 243 diagnosedEgyptian patients with chronic hepatitis C virus, of whom 134 had HCC. Their ages ranged from 37 to 76 years. Patients were recruited from NLI (National Liver institute); Menoufia University; Egypt. Onehundred and twelve healthy subjects were included in the study as control group.The patients were selected during period 2014 to2016.All of patients were have positive of serum hepatitis C virus identified by serology and confirmed by qualitative PCR to detect HCV-RNA .The HCC patients had focal lesion that were detected by ultrasonography and computed tomography (CT)scan .Blood samples were obtained only from patients who gave informed consent .A full history was taken for all patients and control .Peripheral blood samples were collected in two tubes ,the 1st for routine workup including (TLC, Hb, platelets , AST, ALT, Alb, T. bili, Creatinin ,AFP) using commercial assays, and another one for DNA extraction .All investigations were performed in accordancewith the Menoufia University, Health and Human Ethical Clearance Committeeguidelines for Clinical Researches.

2.2. Genotyping of GPX 1 gene

Genomic DNA was extracted from whole blood using QIAampDNA Blood Mini kit (Qiagen, USA)following the manufacturer′s instruction. The extracted DNA was stored at -20ºC until analyzed.Genotyping of the gene wasperformed by polymerase chain reaction–restriction fragmentlength polymorphism (PCR-RFLP) method. Reagents and primerswere provided by Qiagen, USA.

DNA encoding the GPX1 (Pro198Leu) gene polymorphisms was amplified by(PCR).The for each PCR reaction was 12.5µl; PCR reaction ingredients were DreamTaqGreen Master Mix 2x (Fermentas, Thermo Fisher Scientific Inc.), F: 5'TTATGACCGACCCCAAGCTCA3' .andR5':ACAGCAGCACTGCAACTG CC3'and 0.1µg DNA. After a 3 min. denaturing step , amplification wasperformed according to the following cycling profile:94 °C for 60 sec, 56 °C for 60 sec and 72 °C for 60 sec (35cycles). The final elongation step was 5 minat 72 °C. For every reaction, a negative control, in which DNA template was omitted from the amplification mixture ,was included. Amplification product 230 bp was digestedwith restriction enzyme HaeIII. The restricted PCR products wereelectrophoresed through3% ethidium bromide stained agarosegel, and visualized by ultraviolet light.TheGPX1 leucine (L) gene polymorphism produced two products: 148 bp and 82bp while GPX 1 proline(P)gene polymorphism gave three products: 88bp,82bp and 60bp.

For quality control, genotyping of 10% of the sampleswas repeated. Samples were randomly chosen and interpretedblindly by two different observers. The results obtained were identicalto the initial results.

2.3. Detection of HCV RNA

Patients and controls' sera were tested for HCV RNA using RT-PCRmethod.

Statistical analysis

Data were analyzed with statistical Package for the Social Sciences(SPSS version 20.0).According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean±SD,the following tests were used to test differences for significance; Differences between frequencies(qualitative variables)and percentages in groups were compared by Chi-square test. Differences between parametric quatitative independent multiple groups by ANOVA.

Results

Patients were grouped into two groups ; patients with chronic HCV and sub-group diagnosed with HCC , in addition the control group consisted of 112 subjects. Regarding the biochemical data, the haemoglobin, platelets count ,AST, ALT, Albumin , the total bilirubin , creatinin and alph-fetoprotein levels were significantly differences .On the other hand ,TLC showed no difference between patients and controls(Table 1).

The allelic and genotypic frequencies for the GPX1 (Pro198Leu) polymorphismsin controls and patients are shown in Table 2&3. In the HCV group ,the L allele was significantly over-represented compared with control samples : of 486 HCV alleles, 166(34.2%) had the L allele compared to 48/224 (21.4%) control alleles (P=0.0007), also, HCC patients gave the same results when compared with control: of 268 HCC allele , 100(37.3%) had the T allele(P=0.0007).On the other hand, P allele represented high ratio in controls (78.6%) in comparison with HCV (65.8%)(P=0.0002).The results also indicated that the distribution of the all genotypes (PP,PL,LL) were significantly different between the control and HCV group (P =0.000, 0.01,0.004 respectively), the similar results showed with HCC group in compared with control (P =0.000,0.006,0.002 respectively).The results showed that the control subjects had not any case of LL genotype .When evaluating the distribution of combined genotypes(PP+PL) and (PL+LL)in the GPX-1 gene in two groups(HCV patients and controls),we found a highly significant (P = 0.004,0.000 respectively) (Table2).Regarding to combined genotypes (PP+PL)and (PL+LL) in HCC group, also it was found a significant differences compared with control(p=0.002 ,0.000 respectively)Table 3.

Fig 1 .Showed the percent of GPX-1 polymorphisms in genotypes in different groups. We found that the percent of genotype(PP) in control samples was high incidence(57.1%)in comparison with two groups of patients(HCV=37.4%,HCC=32.8%), in contrast the genotype (LL)in patients (HCV=5.8%, HCC=7.5%) was higher than in control samples(0%).

Discussion

During the last decades, a great interest was given to GPX1 as a determinant of cancer risk. Accordingly, the identification of a well-characterized functional polymorphism named p.Pro198Leu (C>T) in GPX1 gene, a lot of studies have been conducted to evaluate the association between p.Pro198Leu polymorphism and risk of cancer development[20].. Therefore, a great interest was given to the association between p.Pro198Leu polymorphism and cancer risk in various populations and a strong association was reported in Denmark, USA, UK and Poland[21,22].Glutathione peroxidase-1, a selenium dependent enzyme, and the proline–leucine substitution makes it less sensitive to stimulation by the addition of selenium [21].. Several research groups have revealed the association between the polymorphism and various diseases caused by the oxidative stress (breast cancer, lung cancer, leukosis, metabolic syndrome, CAD)[17,19,23].Our work was conducted to evaluate the presence of glutathione peroxidase 1 (GPX1) gene polymorphisms in patients with Hepatitic C Virus (HCV) and Hepatocellular Carcinoma(HCC) in Egypt.The present results showed that GPX1 LL genotype had statistically significant differences between patients and controls. This results are agreement with **Ezzikouri et al**[24].who found that patients with the GPX1 LL genotype had more chance to have HCC caused by different etiologies when compared to a healthy control group .In this study,we found a significant differences in GPX1polymorphisms between patients with HCC and controls; the distribution of GPX1 in HCC was (32.8%PP,59.7%PL,7.5%LL) respectively and in controls was(57%PP,42.9%PL,0%LL) .In agreement with this study,**Elelaimy etal**[2].who found the distribution of different GPX1 polymorphisms in HCC patients infected with HCV was (48.0% CC, 39.0% CT and 13.0% TT) respectively and in controls was (53.0% CC, 44.0% CT and 3.0% TT) respectively (P=0.033).Another study done by **Abd El-Ghaffar et al**[25] who found that GPX1 gene polymorphism individuals bearing Leu allele had a 4.9-fold when comparing the HCC group to the control subjects (P=0.001). These results suggested that Pro/Leu genotype might be risky for the development of the inflammation resulting from HCV infection and passes through liver cirrhosis to the development of HCC. Although none of their participants had Leu/Leu genotype, our allelic results suggest that this genotype might possess the highest risk in this process. Further well-designed, multicenter epidemiological studies are necessary to confirm our data in larger subjects and to evaluate the association between the GPX1 polymorphism and cancer risk.

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Biochemistry letters, 13(19) 2018, Pages (235-245)

Table 1: Characterization of patients with HCV, HCC according biochemical data in comparison with control subjects.

Parameter	Control (Mean±S.D) N=112	HCV (Mean±S.D) N=243	HCC (Mean±S.D) N=134	P-Value
TLC	7.2±1.8	6.5±9.3	6.8±7.6	0.713
HB(mmol/L)	12.2±1.8	13±1.9	12.5±1.9	0.006
PLT(1000/mm^3)	273.3±66	209.7±59.4	157.7±91	0.0001
AST(IU/L)	23.4±8	54.7±32.2	70.5±37.2	0.0001
ALT(IU/L)	23.7±32.5	63.4±43.9	62.8±45.1	0.0001
Alb(g/L)	4.2±0.4	4.1±0.6	3.2±0.7	0.0001
T.Bil(mg/dl)	0.7±0.2	0.9±0.6	1.7±2.4	0.0001
Creat(mg/dl)	0.87±0.1	0.9±0.2	1.75±2.4	0.0001
AFP(µg/L)	-	60.4±304	960.3±2300	0.007

Biochemistry letters, 13(19) 2018, Pages (235-245)

Table 2: Allelic and genotypic frequencies of GPX-1genepolymorphisms in control and HCV patients.

***Allelic frequency**

GPX-1gene	Control (%) (n=112/224*)	HCV (%) (n=243/486*)	OR (95%CI)	P-value
Allele				
P	**176(78.6%)**	**320(65.8%)**	**0.525(0.363-0.761)**	**0.0007**
L	**48(21.4%)**	**166(34.2%)**	**1.902(1.313-2.754)**	**0.0007**
Genotype				
PP	**64(57.1)**	**91(37.4)**	**0.449(0.285-0.708)**	**0.000**
PL	**48(42.9)**	**138(56.8)**	**1.752(1.115-2.754)**	**0.010**
LL	**0 (0)**	**14(5.8)**	**1.061(1.029-1.095)**	**0.004**
Combination				
PP+PL	**112(100)**	**229 (94.2)**	**0.645(0.434-0.958)**	**0.004**
PL+LL	**48(42.9)**	**152(62.6)**	**2.227(1.412-3.512)**	**0.000**

Biochemistry letters, 13(19) 2018, Pages (235-245)

Table 3:Allelic and genotypic frequencies of GPX-1 gene polymorphisms in control and HCC patients .

***Allelic frequency**

GPX-1 gene	Control(%) (n=112/224*)	HCC(%) (n=134/268*)	OR(95%CI)	P-value
Allele				
P	**176(78.6%)**	**168(62.7%)**	**0.458(0.305-0.686)**	**0.0002**
L	**48(21.4%)**	**100(37.3%)**	**2.182(1.457-3.288)**	**0.0002**
Genotype				
PP	**64(57.1)**	**44(32.8)**	**0.367(0.218-0.617)**	**0.000**
PL	**48(42.9)**	**80(59.7)**	**1.195(1.187-3.287)**	**0.006**
LL	**0 (0)**	**10(7.5)**	**1.081(1.030-1.134)**	**0.002**
Combination				
PP+PL	**112(100)**	**124(92.5)**	**0.590(0.382-0.911)**	**0.002**
PL+LL	**48(42.9)**	**90(67.2)**	**2.727(1.622-4.586)**	**0.000**

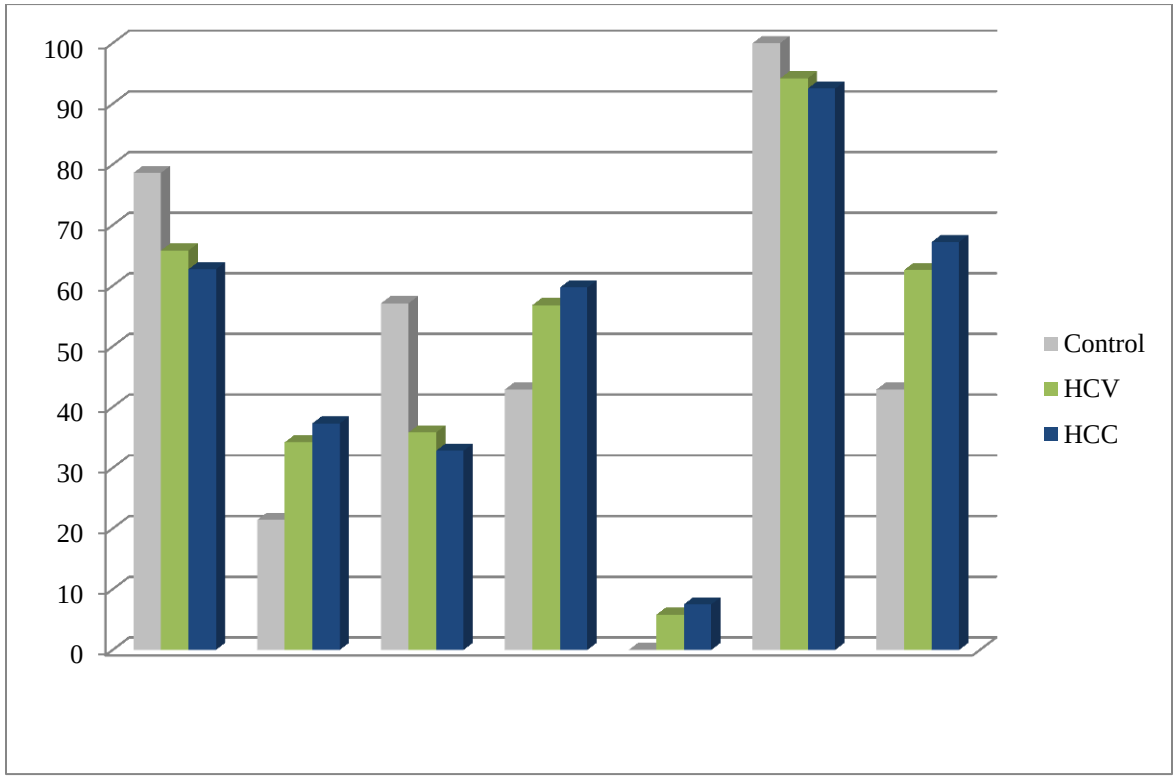

Fig 1:Percent of Allelic and genotypic frequencies of GPX-1 gene polymorphisms in different groups.