ORIGINAL ARTICLE

Genetic and biochemical studies of hepatic carcinoma in the Egyptian population

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Background: Hepatocellular carcinoma (HCC), a deadly malignancy of the liver, is considered the third leading reason behind cancer deaths. It is more frequent in men than in women of ages above 50. Liver disease, leading to liver cirrhosis (LC), is mostly caused by alcoholism abuse, reaction diseases of the liver, or viral hepatitis B or C infection. Interleukin-6 (IL-6) is considered an effective pro-inflammatory cytokine, which plays a crucial role in the host defense mechanism. Its level is higher in HCC patients than in LC cases, indicating that tumor cells increase the production of cytokines. The X-ray repair cross-complementing group 1 (XRCC1) gene is a major DNA repair gene. It acts as a scaffold of various activities that are concerned in the repairing method by interacting with components of base excision repair. This study aims to measure the serum concentrations of IL6 and C-reactive protein (CRP) and investigate whether XRCC1 Arg194Trp and Arg399Gln polymorphisms are related to HCC disease. **Materials and Methods:** Whole-blood DNA was extracted from 123 HCC patients and 123 healthy volunteers. Tetra-primer amplification refractory mutation system was performed in the detection of XRCC1 Arg399Gln and Arg194Trp polymorphisms. **Results:** Serum concentration levels of IL-6 and CRP are significantly higher in patients with HCC than in control subjects. The allelic and genotype frequency distributions of XRCC1 (Arg399Gln and Arg194Trp) are significantly increased in HCC cases compared to healthy volunteers. **Conclusion:** Arg/Gln, Arg/Trp, Gln/Gln, and Trp/Trp genotypes are associated with higher risk HCC than the Arg/Arg genotype.

Keywords: C-reactive protein, hepatic carcinoma, interleukin-6, polymerase chain reaction, X-ray repair cross-complementing group 1

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INTRODUCTION

Hepatocellular carcinoma (HCC) is currently considered the third leading reason behind cancer deaths, globally, wherever the high prevalence of viral hepatitis B and C powerfully causes the event of chronic liver disease and HCC. Recent studies show that it is often recognized in earlier stages as a consequence of the routine screening of patients with wellknown cirrhosis of the liver. Early detection of HCC is also done by the exploitation measurements of alphafetoprotein (AFP) macromolecule and by imaging[1] at which the early detection of the disease is a vital goal that permits the patient to be treated before the enlargement of the neoplasm or its metastasis to distant organs. Unfortunately, in Egypt, the diagnosis is typically detected in a late stage at which neither treatment nor surgery is effective.[2]

HCC has some symptoms that are thought of a good indicator, such as abdominal pain, enlarged abdomen, tenderness (particularly within the upper-right half), easy bruising or hemorrhage, and additionally yellow skin or eyes (jaundice). Other diagnostic tools for HCC include abdominal computed tomography scan, abdominal ultrasound, liver enzymes, and tumor AFP.[3] The risk of HCC incidence in patients with chronic hepatitis C virus increases with the presence of hepatic steatosis when accompanied with complications

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of obesity and diabetes mellitus (DM).[4] Liver cirrhosis (LC) is the major risk factor for HCC, where about 3%–7% of patients with LC detect HCC at an early stage.[5]

AFP is a glycoprotein that is normally produced by the fetal liver, yolk sac, and gastrointestinal tract. AFP serum level is most commonly elevated in HCC. However, such elevation could be seen in many other malignancies in other organs than liver including testicular, bile duct, stomach, and pancreatic and colon cancer. Elevated AFP is also seen with nonmalignant conditions including hepatitis and cirrhosis.[6] It has been used for the surveillance and the diagnosis of HCC in patients with LC.[5]

Interleukin-6 (IL-6) is a pro-inflammatory cytokine that affects much on the immune system cells.[7] Moreover, there is growing evidence indicating that IL-6 is a risk factor for HCC, where high serum level of IL-6 may promote the development of HCC in hepatitis B patients. Consequently, IL-6 could be considered a biomarker for HCC, and an approach based on suppressing IL-6 pathway could be a promising therapeutic strategy for HCC.[8]

C-reactive protein (CRP) is a prototypical acute-phase protein that is produced by the hepatocytes, used to be a prototypical inflammatory cytokine and regulated particularly by IL-6.[9] Studies indicated that CRP could be used as a predictor for HCC as its levels increase hundred-fold, in response to infections and inflammation.[10]

DNA repair has a crucial role in the stability of the DNA genome by repairing the damage of DNA happening due to exogenous and endogenous carcinogenic factors. The ability to repair DNA defects gets affected by any polymorphism that may occur in DNA-repair genes. Such polymorphism may represent a risk factor for any malignancy that may take place as a result of change of base-excision repair (BER) functions. One of the most interesting genes involved in DNA repair is X-ray repair cross-complementing group 1 (XRCC1), which is particularly involved in BER pathway. Studies show that XRCC1-gene variants are associated with a high risk of malignancy, especially HCC.[11]

MATERIALS AND METHODS

The study group included 123 patients with HCCs, with (90 males) and (33 females). Of these 123 cases, only 19 cases were diabetic while the others were nondiabetic. Neither of them has any other complication. The cases were collected randomly from the outpatient of Oncology Department, Faculty of Medicine, Mansoura University, Egypt, during the period from January 2014 to December 2015. Their ages ranged from 49 to 60 years.

Sampling

Venous blood samples (5 ml) were drawn from each of the patients and healthy controls. From these 5 ml, 3 ml was transferred immediately to a clean dry plain tube where the blood was allowed to clot for 10–15 min, at room temperature. Then, the blood is allowed to centrifuge for another 10 min at 3500 rpm to obtain serum for measuring each of IL-6 and CRP. The other 2 ml from the same sample was collected in EDTA tube for the analysis of XRCC1 Arg194Trp and Arg399Gln polymorphisms.

Biochemical markers' determination

IL-6 level was measured using enzyme-linked immunosorbent assay kit according to the method of Bowcock *et al*.[12] CRP concentration was measured by using CRP-latex according to the method of Hanson *et al*.[13]

X-ray repair cross-complementing group 1 genotyping

DNA was isolated from the whole blood according to Bio spin whole-blood genomic DNA extraction kit to BioFlux (Japan). Tetra-primer amplification refractory mutation system was performed for the detection of XRCC1 Arg194Trp (rs1799782) and Arg399Gln (rs25487) polymorphisms, as previously described by Salimi *et al*.[14] The DNA was amplified using specific oligonucleotide primers based on the published sequence.

Each polymerase chain reaction (PCR) reaction mixture was performed in a volume of 20 µl containing 4 µl of generic antisense primer (10 pmol/µl), 10 µl of Green Master mix, mixed with 3 µl of DNA in a thin-walled PCR tube. This mixture was added to 3 µl of specific primer (10 pmol/µl) A or T in separate tubes. The cycling conditions were 6 min at 95°C followed by 35 cycles of 95°C for 30 s, annealing temperature as in Table 1 for 30 s, 72°C for 30 s, and a final cycle 72°C for 6 min. A final holding temperature at 4°C was performed. The PCR products were electrophoresed on 2% agarose gel and visualized using ethidium bromide under ultraviolet illumination.[15] The PCR product for XRCC1 Arg194Trp was detected, with Arg at 297 bp and Trp at 219,

Table 1: Demographic characteristics and biochemical parameters for hepatocellular carcinoma patients and healthy volunteers

	Mean±SE		*P*, OR (CI)
	Control (*n*=31), *n* (%)	Patients (*n*=60), *n* (%)	
Age	49±9.5	60.46±9.5	0.7 (NS), (CI 95%)
Gender			
Female	19 (54.3)	16 (45.7)	0.003, 4.4 (1.7-10.9)
Male	12 (21.4)	44 (78.6)	
Interleukin-6	11.4±5.0	37.3±3.5	<0.0001, (CI 95%)
C-reactive protein	9.1±1.9	23.5±1.4	<0.0001, (CI 95%)

P<0.05 is significant. OR = Odds ratio; CI = Confidence internal; SE = Standard error; NS = Not significant

and then photographed by a digital camera. The primers for analysis were given below.

X-ray repair cross-complementing group 1 Arg194TrpPolymorphism

FO: 5-CGTCCCAGGTAAGCTGTAC-3
RO: 5-CACTCCTATCTATGGGACACAG-3
FI: 5-CGGGGGCTCTCTTCTTCATCC-3
RI: 5-CACCTGGGGATGTCTTGTTGATACA-3.

When the sample has two bands at 297 bp, one with the primer A and the other with the primer T, then it has AT genotype. On the other hand, when the sample has a band at 184 bp, with the A primer, and no band with the G primer, then it has AA genotype. However, if the sample has a band at 219, with T primer, and no band with A primer, then it has TT genotype.

X-ray repair cross-complementing group 1 Arg399Gln polymorphism

FO: 5-ACCAGCTGTGCCTTTGCCAACACC-3
RO: 5-CTGGAGTACCCCAGCCCCTGCC-3
FI: 5-GTCGGCGGCTGCCCTCACA-3
RI: 5-TGGCGTGTGAGGCCTTACCACC-3.

When the sample has two bands at 183 bp, one with the primer A and the other with the primer G, then it has AG genotype. However, if the sample has a band at 183 bp, with the A primer, and no band with the G primer, then it has AA genotype. Finally, if the sample has a band at 140, with G primer, and no band with A primer, then it has GG genotype.

Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows, version 16.0, (SPSS, Inc, Chicago, IL, USA). The mean, standard error, and one-way ANOVA analyses were used to evaluate the significance (*P* value) between the studied variables of the control subjects and HCC patients. A $P < 0.05$ was considered statistically significant. The frequency of genotypes and alleles was compared between HCC patients and healthy controls using the Chi-square or Fisher's exact tests. Student's *t*-test was used for comparison of quantitative variables. The odds ratio (OR) with 95% confidence interval (CI) was calculated to study the association between single nucleotide polymorphisms (SNPs) and HCC disease.

RESULTS

Table 1 shows that there is no statistically significant difference regarding age between both groups. However, there is a significant increase in serum level of IL-6 and CRP in HCC patients ($P < 0.0001$) when compared with the control group.

The genotype and allelic frequency distributions of XRCC1 Arg194Trp and Arg399Gln were studied in HCC cases and control subjects as shown in Table 2. Using the Arg/Arg genotype as the reference genotype, both Trp/Trp genotype (OR of 9.9, 95% CI = 2.9–33.8, $P \leq 0.0001$) and Gln/Gln genotype (OR of 45.55, 95% CI = 6.12–339.1, $P \leq 0.0001$) were significantly elevated in HCC cases compared to controls. The allelic frequencies of XRCC1 Arg194Trp and Arg399Gln polymorphism of the HCC cases were compared with those of the controls using Arg allele as a reference. Both Trp allele (OR = 2.19, 95% CI = 1.54–3.13, $P \leq 0.0001$) and Gln allele (OR = 3.28, 95% CI = 2.28–4.72, $P \leq 0.0001$) are significantly increased in HCC cases compared to control volunteers.

The genotype distributions of XRCC1 codons 194 (Arg > Trp) and 399 (Arg > Gln) in HCC patients with and without family history are shown in Table 3. The results show that there is no significant difference between them.

Table 4 shows that neither the genotype nor the allele of XRCC1 codons 194 (Arg > Trp) and 399 (Arg > Gln) changes significantly in smoker HCC patients in comparison with nonsmoker patients.

Table 5 summarizes the genotype and allele distributions of the XRCC1 194 (Arg > Trp) and 399 (Arg > Gln) polymorphisms in diabetic and nondiabetic HCC patients. Table 5 shows that only the Trp allele was significantly decreased in diabetic HCC group as compared to nondiabetic HCC group ($P = 0.0003$). However, all other genotype and allele frequencies were not significantly

Table 2: X-ray repair cross-complementing Group 1 (Arg194 Trp and Arg399 Gln) gene distributions in both hepatocellular carcinoma cases and healthy volunteers

Polymorphism	Controls (*n*=128)	Patients (*n*=123)	*P*	OR (95% CI)
Arg194 Trp				
Genotypes, *n* (%)				
Arg/Arg	28 (21.9)	0 (0)		Reference
Arg/Trp	97 (75.8)	99 (80.5)		
Trp/Trp	3 (2.3)	24 (19.5)	<0.0001	9.9 (2.9-33.8)
Alleles, *n* (%)				
Arg	153 (59.8)	101 (40.4)		Reference
Trp	103 (40.2)	149 (59.6)	<0.0001	2.19 (1.5-3.1)
Arg399 Gln				
Genotypes, *n* (%)				
Arg/Arg	41 (32)	0 (0)		Reference
Arg/Gln	86 (67.2)	92 (73.6)		
Gln/Gln	1 (0.8)	33 (36.4)	<0.0001	45.6 (6.1-339.1)
Alleles, *n* (%)				
Arg	168 (65.6)	92 (36.8)		Reference
Gln	88 (34.4)	158 (63.2)	<0.0001	3.28 (2.3-4.7)

P<0.05 is significant. OR = Odds ratio; CI = Confidence internal; Arg = Arginine; Gln = Glycine; Trp = Tryptophan

Table 3: Frequency distribution analysis of XRCC1 (Arg194 Trp and Arg399 Gln) gene polymorphisms in hepatocellular carcinoma patients with history

Polymorphism	Without history (*n*=102)	With history (*n*=21)	*P*	OR (95% CI)
Arg194 Trp				
Genotypes, *n* (%)				
Arg/Trp	81 (79.4)	18 (85.7)		Reference
Trp/Trp	21 (20.6)	3 (14.3)	0.76	0.64 (0.17-2.4)
Alleles, *n* (%)				
Arg	81 (39.7)	18 (42.9)		Reference
Trp	123 (60.3)	24 (57.1)	0.73	0.88 (0.45-1.7)
Arg399 Gln				
Genotypes, *n* (%)				
Arg/Gln	76 (74.5)	15 (71.4)		Reference
Gln/Gln	26 (25.5)	6 (28.6)	0.79	1.17 (0.41-3.3)
Alleles, *n* (%)				
Arg	76 (37.3)	15 (35.7)		Reference
Gln	128 (62.8)	27 (64.3)	0.85	1.07 (0.5-2.1)

P<0.05 is significant. OR=Odds ratio; CI=Confidence internal; Arg=Arginine; Gln=Glycine; Trp=Tryptophan

Table 4: Genotype frequencies of X-ray repair cross-complementing Group 1 codons 194 (Arg>Trp) and 399 (Arg>Gln) polymorphisms in smokers and nonsmokers individuals having hepatocellular carcinoma

Polymorphism	Nonsmokers (*n*=101)	Smokers (*n*=22)	*P*	OR (95% CI)
Arg194 Trp				
Genotypes, *n* (%)				
Arg/Trp	81 (80.2)	18 (81.8)		Reference
Trp/Trp	20 (19.8)	4 (18.2)	0.86	0.9 (0.27-2.95)
Alleles, *n* (%)				
Arg	81 (40.1)	18 (40.9)		Reference
Trp	121 (59.9)	26 (59.1)	0.92	0.97 (0.5-1.9)
Arg399 Gln				
Genotypes, *n* (%)				
Arg/Gln	74 (73.3)	17 (77.3)		Reference
Gln/Gln	27 (26.7)	5 (22.7)	0.8	0.8 (0.27-2.4)
Alleles, *n* (%)				
Arg	74 (36.6)	17 (38.6)		Reference
Gln	128 (63.4)	27 (61.4)	0.86	0.92 (0.47-1.8)

P<0.05 is significant. OR=Odds ratio; CI=Confidence internal; Arg=Arginine; Gln=Glycine; Trp=Tryptophan

different when comparing both diabetic and nondiabetic HCC groups together.

DISCUSSION

IL-6 is a pro-inflammatory cytokine that plays a critical role in the host defense mechanism. It has a wide effect on immunity and has dependent pro- and anti-inflammatory feathers that are now regarded as a prominent target for clinical intervention.[16] Studies show that IL-6 may be used as a sensitive and reliable marker in indicating the presence of HCC and other tumors, as its level is highly elevated in high stages of LC and is also correlated with the tumor size and cancer aggressiveness in patients with HCC.[16]

In our study, the level of IL-6 was significantly higher in HCC patients than in healthy volunteers [$P < 0.0001$, Table 2]. This result agrees with that the result shown by Wong *et al*.,[17] who observed that the high serum IL-6 level predates the development of HCC in the Asian population patients and has moderate precision in the prediction of future cancer. It agrees also with Soresi *et al*.,[16] who demonstrated that IL-6 serum levels in HCC Italian patients are higher than in LC patients and controls, which confirms that neoplastic cells produce this type of cytokines.

The present study shows that there is a significant increase in the level of serum CRP in HCC patients compared to control subjects [$P < 0.0001$, Table 1]. Our result is consistent with that of Kinoshita *et al*.,[18] who demonstrated that the pretreatment serum CRP level is associated with tumor progression and reduced liver function and might serve as a separate marker of poor prognosis in HCC patients. Our finding disagrees with Lin *et al*.,[19] who reported that serum CRP is not a good marker for HCC in Taiwan patients, but very high values of CRP in patients with cirrhosis may suggest the presence of a diffuse-type HCC.

Trichopoulos *et al*.[20] observed that plasma CRP level is influenced by diseases associated with long-standing inflammation, confirmed the critical role of inflammation in human cancer, and suggested that plasma CRP level is a potential marker of increased cancer risk.

Pradhan *et al*.[21] observed that elevated levels of CRP and IL-6 predict the development of type 2 DM ($P < 0.001$) and CRP ($P < 0.001$) were significantly higher among cases than among controls. These data support a possible role for inflammation in diabetogenesis levels of IL-6.

The present study analyzed the association of the XRCC1 Arg194Trp A/T SNP and XRCC1 Arg399Gln A/G SNP with HCC in the Egyptian population. Our results demonstrated a strong significant association between both allelic and genotyping distributions of XRCC1 Arg194Trp A/T SNP and XRCC1 Arg399Gln A/G with HCC as shown in Table 2. Table 2 shows that the Trp allele is significantly more frequent in HCC patients than in control subjects (with OR = 2.19, 95% CI = 1.54–3.13, and $P < 0.0001$). This confirms that Trp allele behaves as a dominant variant when using Arg allele as a reference. Thus, Trp allele could be considered as a risk factor of HCC disease.

It is also found that the Gln allele is associated with a significant elevation in HCC cases when

Table 5: Genotype frequencies of X-ray repair cross-complementing Group 1 codons 194 (Arg>Trp) and 399 (Arg>Gln) polymorphisms in diabetic and nondiabetic hepatocellular carcinoma patients

Polymorphism	Nondiabetic (*n*=104)	Diabetic (*n*=19)	*P*	OR (95% CI)
Arg 194 Trp				
Genotypes, *n* (%)				
Arg/Trp	85 (81.7)	14 (73.7)		Reference
Trp/Trp	19 (18.3)	5 (26.3)	0.53	1.6 (0.51-4.97)
Alleles, *n* (%)				
Arg	85 (40.9)	14 (87.5)		Reference
Trp	123 (59.1)	2 (12.5)	0.0003	0.099 (0.022-0.45)
Arg399 Gln				
Genotypes, *n* (%)				
Arg/Gln	79 (76.0)	12 (63.2)		Reference
Gln/Gln	25 (24.0)	7 (36.8)	0.26	1.8 (0.65-5.2)
Alleles, *n* (%)				
Arg	79 (37.98)	12 (31.6)		Reference
Gln	129 (62.02)	26 (68.4)	0.58	1.33 (0.6-2.8)

P<0.05 is significant. OR=Odds ratio; CI=Confidence internal; Arg=Arginine; Gln=Glycine; Trp=Tryptophan

compared to control volunteers using the Arg allele as a reference (where OR = 3.28, 95% CI = 2.28–4.72, and $P < 0.0001$). Using the Arg/Arg genotype as the reference genotype, it is found that Trp/Trp genotype was significantly elevated in HCC cases in comparison with control subjects [OR of 9.9, 95% CI = 2.9–33.8, and $P < 0.0001$, Table 2]. This result agrees with Wong *et al*.,[17] who suggested that the genotypes of XRCC1 Trp/Trp might be the risk genotype for lung cancer in Chinese population. Table 2 shows further that Gln/Gln genotype is highly significantly increased in HCC patients than healthy controls (OR of 45.55, 95% CI = 6.12–339.1, and $P < 0.0001$).

Our study data also demonstrated that the Gln allele frequency is significantly increased in HCC patients when compared healthy subjects (with OR = 3.28, 95% CI = 2.28–4.72, and $P < 0.0001$), and therefore, it might be considered a risk factor for the incidence of HCC. This is in congruence with the conclusion of Li *et al*.[11] among East Asian population ($P = 0.066$) and Pan *et al*.,[22] who demonstrated that XRCC1 codon Arg/Gln is associated with an increased risk of HCC in the Chinese population, especially for patients above 50-year-old or with drinking habits. Conversely, Zeng *et al*.[23] and Bo *et al*.[24] reported that XRCC1 codon Arg/Gln polymorphisms, in the Chinese population, are not associated with HCC risk ($P = 0.56$).

To the best of our knowledge, this study is the first to report that XRCC1 codon Arg/Trp genotype is associated with a significantly increased risk of HCC in Egyptian population [$P < 0.0001$, Table 2].

Guo *et al*.[25] and Yang *et al*.[26] reported that XRCC1 Arg194Trp gene polymorphism in the Indian and Chinese populations may not be associated with the risk of HCC ($P = 0.366$). Their result is in accordance with that of Bo *et al*.[24] on Chinese population ($P = 0.86$). The differences between their result and ours may be due to race difference, age, sex, population sample size, and other environmental factors. Furthermore, this contradiction may be related to the difference in the origin of patients, their susceptibility, and handling of specimens as well as time of measuring from the onset of the disease.

The influence of Egyptian HCC family history, smoking, and having DM with the risk of HCC and also the association with XRCC1 codons Arg194Trp and Arg399Gln polymorphisms have been investigated in this work. Our observations show that there is no association between HCC family history and smoking and both XRCC1 polymorphisms in our HCC patients group [Tables 3 and 4]. Our observations show further that, except the Trp allele in the codon Arg194Trp which has a significant association, having DM does not have an influence on the frequency distribution of XRCC1 genotypes in HCC Egyptian patients. Yuan *et al*.[27] found that alcohol consumption, use of tobacco, and DM history could be considered as independent predictors of HCC risk in Hispanic and non-Hispanic Whites and Blacks in Los Angeles County.

Our findings are conflicting with those of El-Serag *et al*.[28] and Wanfg *et al*.,[29] who found that having DM is significantly associated with the increased risk of HCC. Such conflict between our observations and those reported by others may be attributed to the difference in race, gender, and duration of the disease.

CONCLUSIONS

XRCC1 polymorphisms are still a major topic in liver cancer research. Arg/Gln, Arg/Trp, Gln/Gln, and Trp/Trp genotypes are associated with higher risk HCC than the Arg/Arg genotype in Egyptian population. Smoking and family history of HCC patients play no role in XRCC1 polymorphisms. Moreover, the significant difference in the levels of IL-6 and CRP between HCC patients and healthy volunteers indicates that both IL-6 and CRP may be considered diagnostic biomarkers for predicting HCC.

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Conflicts of interest

There are no conflicts of interest.

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