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The relationship between the IL-1β 31gene polymorphism and HCC in Egyptian patients

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Keywords

- *IL-1 β*
- *Polymorphism*
- *viral hepatitis*
- *hepatocellular carcinoma*

Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and has an increasing incidence in Egypt. Hepatitis virus (HCV and HBV) are the major risk factors for developing HCC. IL-1β, a proinflammatory cytokine has been suggested to affect the hepatic carcinogenesis. **Aim of the work**: Was to evaluate the role of IL-1β polymorphism in the occurrence of HCC on top of viral versus none-viral etiology. **Patients and Methods**: The study included 178 patients with HCC (74 with HCV, 48 with HBV, and 56 without hepatitis virus) and 90 healthy volunteers represented the control group. The polymorphism in IL-1β –31 gene was investigated by polymerase chain reaction and restriction fragment length polymorphism. Quantitative determination of IL-1β serum level was performed using ELISA technique. **Results**: The frequency of TT genotype was higher in HCC patients with HCV when compared to HCC patients without viral hepatitis, and the control group (43.2%, 25% and 26.7%, respectively). We observed that the dominant model (TT and CT genotypes) were associated with increased HCC risk in HCV or HBV patients compared to the control group with odd ratio and 95%CI of 2.64 (1.3-5.3) and 2.77 (1.2-6.2) respectively. In addition, the T allele was more frequent in HCC patients with HCV or HBV when compared to none viral HCC and the control group (60.8%, 56.2%, 42.9 and 42.2%, respectively). Serum IL-1β level was elevated in all HCC groups as compared to the control group ($p<0.05$). Serum level of IL-1β was considerably increased in individuals with TT genotype or T allele as compared to other genotypes and allele ($p<0.0001$). **Conclusion**: IL-1β TT genotype and T allele which are associated with high blood IL-1β level may increase the risk of HCC in patients with chronic viral hepatitis.

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Introduction

Hepatocellular carcinoma (HCC) represents the 5th common malignancy and the 2nd common cause of cancer related death worldwide (1). World Health Organization expected that the rising HCC burden will persist until 2030 (2). The severity of liver malignancy in Egypt was reported by Ibrahim et al. (3), it is classified as the first cancer in males (33.6%) and the second after breast cancer in women (13.5%).

Khattab et al. (4) reported that the highest incidence of hepatitis C virus (HCV) in the world was found in Egypt with an average 13.8% in the general population. The propagation of infection with HBV in Egypt is 4.5% and about 2-3 millions of Egyptian individuals are considered chronic carriers of HBV (5). These percentages reflect the national level epidemic of viral hepatitis in Egypt (6).

Hepatic carcinogenesis is multistep and multi-factorial process in which there are many risk factors as chronic hepatitis C or B infection. However, the most implicated factors are the inflammation and host genetic elements. Inflammation stimulates angiogenesis, DNA damage and malignant cell growth (7). Other risk factors to hepatocarcinogenesis include dietary aflatoxin, pesticides, chronic abuse of alcohol, metabolic and some hereditary disorders as hereditary hemochromatosis and alpha1-antitrypsin deficiency (7, 8).

Interleukin-1beta (IL-1β), a proinflammatory cytokine that is synthesized by liver macrophages and implicated in the course of HCV (9). High blood level of IL-1β is found in hepatitis, liver fibrosis, and liver cirrhosis and HCC patients (10). IL1β gene is highly polymorphic and located at position 1 on the long arm of chromosome 2 (11). One of these polymorphisms present in the promoter region at −31 positions (12). IL-1β gene −31C/T substitution is located in the TATA box and affecting the binding of transcription initiation factors. Consequently, this affects the IL-1β transcription activity and modulates its expression (13). Liver cirrhosis and end stage liver disease were found to be associated with gene polymorphisms of IL-1β (14). However, the relationship between IL-1β gene polymorphisms and the occurrence of cancers has been examined and the results were controversial (15). So the present study investigated the role of IL-1β −31 C/T polymorphism in the occurrence of HCC in Egyptian patients with viral hepatitis (HCV, HBV) versus none-viral cause.

Subject and Methods

Study population

A case-control study was done between 2014 and 2016 and included 178 HCC patients and 90 healthy volunteers. The patients were enrolled from those attending Oncology Center and Internal Medicine Department, Mansoura University Hospital. All participants were undergone to complete medical history taking and clinical examination. HCC diagnosis was based on histopathology or on two imaging modalities if histopathological examination not done. Imaging modalities include magnetic resonance imaging, computed tomography or contrast-enhanced ultrasound showing an enhancing vascular mass of more than 2 cm (16). Serological markers for hepatitis B and C were assayed by ELISA (Access

BIO- RAD Co., France) and positive cases for HCV antibodies were confirmed by qualitative viral RNA detection using AmpliPrep/COBAS TaqMan HCV Qualitative test version 2.0 with detection limit of 15 IU/mL (Roche Molecular Diagnostic, Branchburg, NJ, USA) according to guidance of manufacturer.The blood samples were withdrawn before any treatment intervention. Exclusion criteria were: diabetes mellitus, chronic renal failure, coronary artery disease, other malignancy, end stage and autoimmune liver disease. The study was done with the agreement of Faculty of Medicine, Mansoura University local ethics committee and in accordance with the General Assembly of the World Medical Association Declaration of Helsinki (Reference number R/17.02.97).Written consent was obtained from each participant.

The participants were divided into four groups as follow:

Group I: included 90 healthy volunteers as normal control

Group II: included 74 HCC patients with HCV: they were seropositive for hepatitis C virus antibodies and HCV-RNA.

Group III: included 48 HCC patients with HBV: they were seropositive for hepatitis B markers.

Group IV: included 56 HCC patients with none B none C (HCC due to none viral etiology): They were sero-negative for hepatitis B markers, HCV antibodies and HCV-RNA

Demographic data of the studied groups are presented in Table (1).

Samples:

Whole blood sample (5 ml) was drawn from each participant. One ml collected in EDTA containing tubes for subsequent fresh DNA extraction and the extracted DNA was stored at −30 °C for later IL-1β gene polymorphism. The rest of the sample (4ml) has been collected in plain tubes for serum separation. The serum was used for the assay of IL-1β and alpha fetoprotein (AFP) level.

Methods

Extraction of genomic DNA and Genotyping of IL-1β −31C/T gene:

Genomic DNA was extracted from whole blood samples by DNA purification kit (Qiagen GmbH, Cat No.51104, Hidden, Germany). Traditional PCR was used for amplification of IL-1β −31C/T gene as previously described by Hwang et al. (17). Two primers sequences were designed for gene amplification (F: 5'-AGAAGCTTCCACCAATACTC-3', R: 5'-AGAAGCTTCCACCAATACTC- 3'). Amplification was done according to the following temperature program: initial denaturation for one minute at 95 °C then 36 cycles of denaturation for 45 seconds at 94°C, annealing for 50 seconds at 54°C, extension at 72°C for 60 seconds. Finally 7 minutes at 72 °C for extension stage. The resulting PCR products were 448 bp lengths. For genotyping of −31C/T gene, PCR products were digested using Alu I (Invitrogen. Cat. No. 45200-029, Life Technologies Corporation, China) according to the protocol of Okamoto et al (18). The products were detected using 3 % agarose gel electrophoresis. The mutant T allele: 247, 97, 79, 20 and 5 bp and the wild C allele: 344, 79, 20 and 5 bp as shown in figure (1).The small sized bands 79, 20 and 5 Bp do not appear in the gel.

Determination of the IL-1β serum level

Serum IL-1β level was assayed by quantitative sandwich enzyme labelled immune assay (Max Human, Cat. No. EI2200-1. USA) according to commandment of manufacturer. The detection range is 0.5 – 80 pg/ml. The optical density of each sample was determined using ELISA reader (Huma, Germany) set at wavelength 450 nm with correction at 570 nm.

Determination of AFP serum level

Serum AFP level was assayed by quantitative sandwich enzyme labelled immune assay (Quantikine, Cat. No. DAFP00, USA) according to guidance of manufacturer. The detection range is 0.31 to 20 µg/ml. The optical density of each sample was determined using plate reader (Huma, Germany) set at 450 nm with correction at 570 nm.

Statistical Analysis

Excel program and SPSS version 22 were used to analyze the resulting data. Statistical significance was detected between different studied groups. For analysis of quantitative data, Mann-Whitney test significance was used to compare two groups, and Kruskal Wallis test was used to compare more than two groups. To compare qualitative data, χ^2 test was used. $P< 0.05$ was considered statistically significant at 95% confidence interval. The frequencies of genotypes and alleles in cancerous patients and healthy controls were tested for Hardy–Weinberg Equilibrium.

Results

Serum value of AFP

There was significant increase of AFP serum level in all HCC groups compared to control, but no significant difference was found between different HCC groups ($p>0.05$). Data are shown in table (1).

Serum value of IL-1β:

There was a significant increase of IL-1β serum level in all HCC groups compared to control also, IL-1β serum level was increased in HCC with HCV or HBV when compared to HCC without viral cause. Data are shown in table (1).

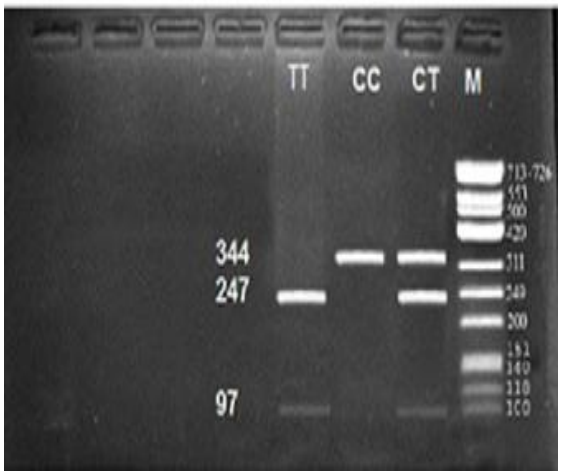

Figure (1): Agarose gel electrophoresis showing the enzymatic digestion of IL-1β −31C/T gene polymorphism for different groups studied; Lane M: ΦX174 DNA/Hinf I marker, lane CT genotype (344, 247 and 97), CC genotype (344), and lane TT genotype (247 and 97).

Table (1): AFP and IL-1β level in the control group and HCC group:

	Control group (number=90)	HCC group		
		HCC with HCV (number=74)	HCC with HBV (number=48)	HCC without hepatitis (number=56)
Age (year)				
Median	59	61	58.5	68
Interquartile Range	47-71	48-62	52- 67	58-72
Sex no (%)				
Male	56 (62.2%)	50 (67.5%)	30 (62.5%)	24 (42.9%)
Female	34 (37.8%)	24 (32.4%)	18 (37.5%)	32 (57.1%)
Tumor size (cm)				
Median	0	3.1	2.95	2.95
Interquartile Range		1.3-4.2	1.6-4.5	1.5-4.2
AFP (µg/ml)				
Median	10.2	1634[a]	1977.5[a]	524.5[a]
Interquartile Range	4.75-15.7	312-3245	1085.3-3124.5	213.3-2913.8
IL-1β (pg/ml)				
Median	70	398[a,b]	407[a,b]	287[a]
Interquartile Range	64-74	374-437	369.3-444.5	255.3-334.5

[a] Significant difference from control group ($p<0.05$).
[b] Significant difference from HCC without hepatitis ($p<0.05$).

Table (2): Genotype distribution and allele frequency of the IL-1β −31 polymorphism in control group and HCC group:

	Control group (number =90)	HCC group		
		HCC with HCV (number =74)	HCC with HBV (number =48)	HCC without hepatitis (number =56)
Genotypes				
TT	24 (26.7%)	32 (43.2%)[a,b]	16 (33.3%)	14 (25.0%)
TC	28 (31.1%)	26 (35.1%)	22 (45.8%)	20 (35.7%)
CC	38 (42.2%)	16 (21.6%)[a,b]	10 (20.8%)[a,b]	22 (39.3%)
Alleles				
T allele	76 (42.2%)	90 (60.8%)[a,b]	54 (56.2%)[a]	48 (42.9%)
C allele	104 (57.8%)	58 (39.2%)[b]	42 (43.8%)[a]	64 (57.1%)

[a] Significant difference from control group ($p<0.05$).
[b] Significant difference from HCC without hepatitis ($p<0.05$).

Table (3): Odds ratio for IL-1β genotypes and alleles and associated risk of HCC

	Control group (n=90)	HCC group		
		HCC with HCV (number =74)	HCC with HBV (number =48)	HCC without hepatitis (number =56)
Dominant model				
CC	38 (42%)	16(21%)	10(20%)	22(40%)
CT+TT	52(58%)	58(79%)	38(80%)	34(60%)
Odds ratio	Reference	2.64	2.77	1.12
95% CI	-	1.3-5.3	1.2-6.2	0.57-2.23
p-value	-	0.005*	0.01*	0.72
Recessive model				
TT	24(27%)	32(43%)	16(33%)	14(25%)
CT+CC	66(73%)	42(57%)	32(67%)	42(75%)
Odds ratio	Reference	2.09	1.37	.91
95% CI	-	1.08-4.03	0.64-2.94	0.42-1.96
p-value	-	0.02*	0.4	0.82
Alleles				
T allele	76 (42.2%)	90 (60.8%)	54 (56.2%)	48 (42.9%)
C allele	104 (57.8%)	58 (39.2%)	42 (43.8%)	64 (57.1%)
Odds ratio	Reference	2.12	1.75	1.02
95% CI	-	1.36-3.3	1.06-2.9	0.63-1.6
p-value	-	0.0009*	0.02*	0.9

* Significant difference from control group (p<0.05).

Table (4): Serum AFP and IL-1β levels in different genotypes and alleles of IL-1β −31 C/T gene among all participants:

Parameter	IL-1β genotypes			P Value	IL-1β alleles		p value
	TT number=86	CT Number=96	CC number=86		T number=268	C number=268	
AFP (µg/ml) Median Interquartile Range	353 20-2566	545 14-2776.5	186 12-1634	0.157	500 18-2566	344 13.3-2354	0.286
IL-1β (pg/ml) Median Interquartile Range	402 75-443	327.5 75- 388.5	242 71-353	<0001*	374 75-422	274 73-374	0.002*

*= significant p value (p<0.05).

IL-1β 31 genotype and allele

There was significant increase in the frequency of TT genotype in HCC patients with HCV when compared to HCC without viral hepatitis, and control group (43.2%, 25% and 26.7%, respectively). On other hand, the CC genotype was more frequent in both control and HCC patients without viral hepatitis groups when compared to HCC with HCV or HBV (42.2%, 39.3%, 21.6%, and 20.8%, respectively). In addition, the T allele exhibited higher frequency in HCC patients with HCV or HBV than none viral HCC and the control group (60.8%, 56.2%, 42.9 and 42.2%, respectively) (Table 2).

Odds ratio for IL-1beta genotypes and alleles

Regarding frequency of IL-1 β alleles and genotypes and associated HCC risk; where mutant

T allele showed significant increase in HCC patients with HCV or HBV as compared to the control group with increased risk of HCC development in hepatitis groups, where ORs and 95% CI of 2.12 (1.36-3.3) and 1.75 (1.06-2.9) respectively. On the other hand, there was no significant difference in the frequency of T allele between none viral HCC patients and the control group with no increased HCC risk in none viral patients. In addition, the dominant model (TT and CT) genotypes were associated with increased HCC risk in HCV or HBV patients compared to the control groups with ORs and 95% CI of 2.64(1.3-5.3) and 2.77 (1.2-6.2) respectively. On the other hand, the dominant model (TT and CT) genotypes showed no significant difference between HCC patients with none viral cause and control with no increased HCC risk in this group (Table 3).

IL-1β level in different genotypes and alleles among all participants

There was highly significant increase of IL-1β level in individuals with T allele and TT genotype when compared with other allele and genotypes ($p<0.0001$). AFP serum level did not show significant difference between different studied alleles and genotypes among all participants (table 4).

Discussion

Hepatocellular carcinoma is an inflammation related cancer as most cases develop on top of inflammation as well as cirrhosis (10). Proinflammatory cytokines such as IL-1β and genetic alterations stimulate the hepatocarcinogenesis through growth signaling, angiogenesis and invasiveness (19). Hepatitis viruses are responsible for about 70% to 80% of HCC etiology (20). They contribute to oncogenic transformation (21) through induction of chronic inflammation (22). In this study, we evaluated the relationship between IL-1β −31C/T gene polymorphisms and the incidence of HCC in Egyptian patients due to different etiology as HCV, HBV and none viral causes. It was found that the frequency of T allele in HCC with HCV or HBV is more than in control group. Regarding the genotype, HCC with HCV group showed the highest frequency of IL-1β 31 TT genotype. These results are in accordance to that reported by Yeo et al. (23) and Wang et al. (24). Also, the −31 CT genotype of IL-1β showed a higher frequency in HCC with HBV group, that in agreement to Chen et al. (25), however, among three genotypes, the wild type CC showed higher frequency in HCC without viral cause than other genotypes. The findings of the current study are supported by the result of Tarhuni et al. (26) who reported that the development of viral based HCC is increased with the presence of the IL-1β−31 T allele (TT > CT> CC), also the genetic variation modifies the individuals' responses to cancer-related infection (27). Thus, IL-1β−31C/T polymorphism contributes to genetic susceptibility for hepatitis virus related HCC as reported by He and his coworkers (15).

Regarding the serum level of IL-1β, there is considerable increase in patients with T allele than C allele as variant T allele is associated with higher IL-1β expression and production than C allele. This is in accordance with the study done by Chang et al. (28) and can be explained on the base of IL-1β −31C/T polymorphism increases the binding activity of the transcription initiation factors (29) as

C/EBP β, HMGB1, PU.1 and the TATA box binding protein (30). The synergistic activity of the C/EBP β and PU.1 transcription factors is increased by a single base change in the IL-1β promoter leading to increase IL-1β expression (31). High IL-1β expression leads to increased risk of cancer (32). On contrary, Hamacher et al. (33) found that C allele is associated with higher secretion of IL-1β than T allele in pancreatic cell lines. High IL-1β in HCC with HCV is attributed to inflammasome-stimulated IL-1β production through NALP3 signaling (34). However, HBV induces IL-1β release from liver macrophages (35). These could explain the finding of the current work regarding significantly higher IL-1β level in viral related HCC in comparison to none viral HCC.

IL-1β mediates liver inflammation by inducing the expression of proinflammatory genes, recruiting immune cells and modulating the cellular immunity (36). Hepatocytes, monocytes and stellate cells are multiple sources of IL-1β in HCC microenvironment (37). High inflammatory IL-β level induces hepatocarcinogenesis by several mechanisms. First it activates the oncoprotein Gankyrin through IL-1β receptor associated kinase-1 signaling cascade (10). Gankyrin accelerates the expansion of tumor-initiating cells by preventing the octamer-binding transcription factor 4 degradation (38). Second it induces mutations of cancer associated genes such as TP53 (which encodes p53) and Myc (39). Induction of Myc-driven genes signals the transformation from dysplastic nodules to HCC (40). Third it down regulates the expression of mismatch repair proteins genes MSH2 and MSH6 (41). However, IL-β promotes tumor invasiveness (42) and angiogenesis (20) by increasing COX, VEGF and nitric oxide (43). Moreover, it participate in metastasis through the induction of oncogenic cytokines GRO-a, IL-6 as well as IL-8 from malignant cells (44). Taken together, IL-1β −31T/C polymorphism by modulating IL-1β production is candidate genetic factor that link chronic inflammation and hepatocarcinogenesis (45).

Conclusion:

IL-1β TT genotype and T allele which are associated with high blood IL-1β level may increase the risk of HCC in chronic viral hepatitis patients. Screening of these polymorphisms and surveillance of high risk group will allow early detection and treatment of HCC. Continuous treatment of HCV, the main cause of HCC in Egypt could much decrease the future incidence of HCC in Egyptian population. Further studies are recommended on large scale to confirm or reject these results and to avoid any bias of data.

Disclosure statement:

The authors state no conflicts of interest and they alone are responsible for the content and writing of the paper.

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