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Genetic variants in IL-6 and IL-10 genes and susceptibility to hepatocellular carcinoma in HCV infected patients

Ikram Sghaier [a,*], Leila Mouelhi [b], Noor A. Rabia [c], Bano R. Alsaleh [c], Ezzedine Ghazoueni [d], Wassim Y. Almawi [c], Besma Yacoubi Loueslati [a]

[a] University of Tunis El Manar, Faculty of Sciences of Tunis, Laboratory of Mycology, Pathologies and Biomarkers: LR16ES05, 2092, Tunisia
[b] Charles Nicolle Hospital, Hepato-Gastro-Enterology Department, Tunis, Tunisia
[c] Department of Medical Biochemistry, Arabian Gulf University, Manama, Bahrain
[d] Military Hospital of Tunis, Laboratory of Immunology, Tunis, Tunisia

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ABSTRACT

Background: Hepatitis C virus (HCV) infection is the major cause of hepatocellular carcinoma (HCC), a common primary liver malignancy, and the third leading cause of cancer-related death. The HCC risk increases with the severity of liver inflammation, and the clinical course of HCV infection depends on a balance between pro- and anti-inflammatory cytokines. The former includes interleukin (IL)-6, while the latter includes IL-10. However, the exact pathogenic mechanisms underlying IL-6 and IL-10 effects remain unclear.

Methods: The present study evaluated 174 chronic HCV Tunisian patients. Polymorphisms of *IL-6* (rs1880242, rs1474847, rs2069840, rs1800797, rs1800796, rs2069845, rs2069827, rs1474348, rs1800795), and *IL-10* (rs1800896, rs1800871, rs1800872, rs1554286, rs1878672, rs1518111) were determined by real-time PCR.

Results: Notable differences between chronic HCV-infected patients and HCC patients were observed for the three *IL-10* SNPs; rs1800871 (−819T/C), rs1800872 (−592A/C), and rs1878672. Carriage of *IL-6* rs1800796 G/G genotype, *IL-6* rs1474358 C-allele, and *IL-6* rs1800797 A-allele was more frequent in chronic HCV-infected patients than in HCC patients. On the other hand, *IL-6* rs1474358 GG genotype had a favourable factor for HCC establishment.

Conclusion: *IL-10* and *IL-6* SNPs markedly influence the clinical outcomes of HCV infection. These SNPs could be used as biomarkers for early detection and molecular therapy for preventing HCC, and prognostic factors for predicting the clinical outcomes of HCC.

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1. Introduction

The incidence of HCV and related complications continue to rise globally, and World Health Organization (WHO) reporting an estimated 3% prevalence worldwide [1,2]. Hepatocellular carcinoma (HCC) is the primary liver malignancy, and the third leading cause of cancer-related deaths worldwide [2]. Chronic HCV infection is associated with variable outcome, ranging from simple hepatic damage, to cirrhosis, and HCC [3]. Most HCV-related HCC cases are found in developing countries [4], and the progression and the development of HCC correlates with HCV infection, predominantly in cirrhosis. Accordingly, routine examination and assessment, including HCV genotype and viral load, will improve the prognosis of HCV infection.

The course of HCV infection is influenced by modifiable and non-modifiable factors, such as duration of infection, gender, alcohol consumption, age, and insulin resistance [5]. Despite the identification of these and related factors, the exact cause underlying most HCV infection cases remain unexplained, suggesting the contribution of other factors, in particular the immune status of the host. A large numbers of single nucleotide polymorphisms (SNPs) in both pro- and anti-inflammatory cytokine genes were previously associated with the outcome of chronic HCV infection [6], including interleukin (IL)-6, a pleiotropic cytokine with a key role in inflammation [7], and innate adaptive immunity [8]. As carcinogenesis is accompanied by a state of low-grade inflammation, a key role for altered IL-6 signalling in human cancers were reported previous reports linking [9–11].

* Corresponding author.
E-mail address: sghaiere.ikram@gmail.com (I. Sghaier).

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Recently, IL-6 was shown to drive the differentiation on naïve Th0 cells to the Th17 cell lineage [12,13]. This expands the role of IL-6, from involvement in innate immunity to its significant contribution to chronic inflammation accompanying disease process. IL-6 is a pleiotropic cytokine expressed by many cell types, and regulates several inflammatory processes, haematopoiesis and immune regulation. Dysregulated IL-6 expression was often seen in cancer, and a role for IL-6 as a prognostic biomarker for breast and prostate cancer was suggested [14]. Elevation in IL-6 serum levels correlated with advanced stages of cancer, and was linked with poor prognosis. The pleiotropy and redundant functions of IL-6 underscores an important signalling axis in oncogenic transformations.

On the other hand, IL-10 is an anti-inflammatory cytokine, which downregulates the synthesis of pro-inflammatory cytokines, including IL-6, and modulates hepatic fibrogenesis [15]. Several study addressed the possible contribution of *IL-10* promoter polymorphisms to HCV outcomes. IL-10 plays a key role in the oncogenic and metastatic ability of neoplasms [16], exemplified by the reported increases in IL-10 levels in cancer patients, including those with HCC [17,18], where it was related to the disease prognosis. Several cell types, including T cells, B cells, monocytes, macrophages, mast cells, granulocytes, dendritic cells and keratinocytes, produce IL-10. Tumor cells and tumor-infiltrating macrophages are also major IL-10 secreting cells [19,20]. IL-10 exerts immunosuppressive effect on antigen presentation, cell maturation and differentiation, thereby allowing evasion of tumor cells of immune recognition [21]. This indicates that IL-6 and IL-10 drive tumor progression by dampening anti-tumor immunity, and by precipitating by state of inflammation, and escape of apoptotic processes.

The aim of this study was to determine whether specific *IL-10* genotypes influence the outcomes of HCV infection in Tunisian patients. The second objective was to examine the possible association of *IL-6* promoter polymorphisms with the outcomes of chronic HCV infection.

2. Materials and methods

2.1. Study population

Blood samples were collected from 174 Tunisian patients infected with HCV, who were divided into three groups. Group1 (G1) consisted of patients infected with chronic hepatitis C (CHC), and positive for both anti-HCV Ab, and HCV RNA (assessed by RT-PCR). Group 2 (G2) comprised patients infected with cirrhosis (LC), but with no histological evidence of cancer. Lastly, Group 3 (G3) included patients with established HCC, which was confirmed by pathology, computer tomography (CT) imaging, and serum alpha-fetoprotein (AFP). All the studied groups were free from heart diseases, kidney diseases, muscle disorders, pancreatitis, and concurrent HBV, HDV and HIV infections. Patients were tested for aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, HIV (anti-HIV), hepatitis B (HBsAg, anti-HBs, anti-HBc), hepatitis C (anti HCV) serology. In case of reactive ant-HCV positivity, HCV-RNA and viral genotyping tests were performed. All patients gave informed consent for participating in this study.

2.2. DNA extraction

Peripheral venous blood was collected from participants in EDTA-containing tubes. Total genomic DNA was prepared by the mini-spin column method, using QIAamp® DNA blood Mini Kit, according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany).

2.3. IL-10 and IL-6 Single Nucleotide Polymorphisms (SNPs)

We selected six *IL-10* SNPs (rs1800896, rs1800871, rs1800872, rs1554286, rs1878672, rs1518111), with minor allele frequency (MAF) >5%, and nine *IL-6* SNPs (rs1880242, rs1474847, rs2069840, rs1800797, rs1800796, rs2069845, rs2069827, rs1474348, rs1800795) previously associated with HCV outcome [23,24], which were previously reported in the literature with clinical relevance using NCBI Gene SNP Geneview. Genotyping was performed by allelic (VIC- and FAM-labelled) discrimination method, using assay-on-demand TaqMan assays, which were ordered from Applied Biosystems (Foster City, CA). The reaction was performed in 6 µl volume on StepOne/StepOne Plus real-time PCR systems, according to manufacturer's instructions (Applied Biosystems). Genotypes were assigned from output of clusters, with homozygous major (blue), heterozygous (green), and homozygous minor (red) genotypes color-coded. Replicate blinded quality control samples were included to assess reproducibility of the genotyping reaction and concordance was >99%.

2.4. Statistical analysis

Statistical analysis and graphing were performed with R and R studio program, version 3.2.3. The characteristics of study subjects were reported as medians, or/and relative frequencies (%). The effect of *IL-10* and *IL-6* polymorphisms on gene expression and clinical data were evaluated by setting homozygous major allele genotype as reference; subsequent analyses were done using one-way ANOVA. Chi-square test (or Fisher's exact test for low numbers) was used in comparing categorical data. Logistic regression analysis was used in analyzing the independent contribution of key covariates with HCV outcomes; $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

The general characteristics of the three patient groups included in this study are shown in Fig. 1. There was no significant difference observed between three groups in terms of HCV genotype, and weight. Despite of the lack of association of gender and HCC, it was reported that men are usually more susceptible to HCC than women, partly because of different social habits (alcohol and cigarettes) [25]. Liver transaminase levels, stage of fibrosis, grade of necro-inflammatory activity, and AFP levels were highest in the HCC group, while it was the lowest in patients with chronic infection ($P < 0.001$). In addition, age at the time of infection markedly affected the rate of fibrosis and HCC development, with significant differences between the three groups ($P = 0.001$).

3.2. IL-10 polymorphisms and HCV outcomes

Six *IL-10* SNPs were studied. Table 1 summarizes the frequencies of the genotypic profiles of the tested SNPs, first between patients with chronic infection and cirrhosis, and second between patients with chronic infection and the HCC group. Univariate, and later multivariate analysis, confirmed significant differences between G1 and G3 in the distribution of three of the six tested *IL-10* SNPs: rs1800871 (−819 T/C), rs1800872 (−592 A/C) and rs1878672.

The distribution of rs1800871 T/T genotype was significantly different between CHC and HCC patients [$P = 0.02$, OR (95%CI) =

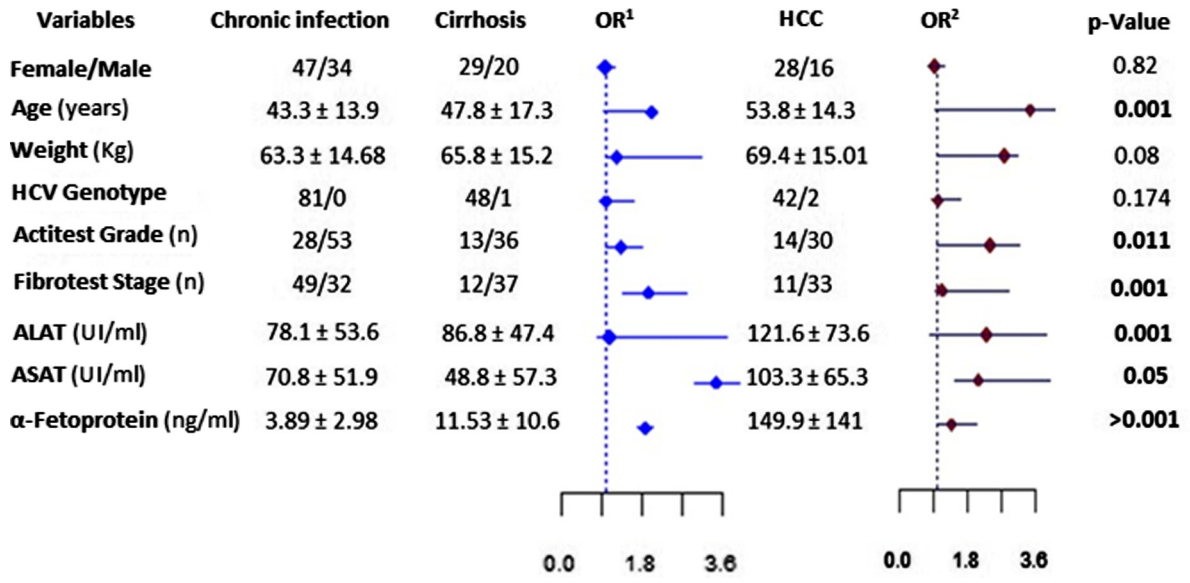

Fig. 1. Clinical characteristics of the study population.

Table 1
IL-10 polymorphisms and HCV infection outcomes.

SNPs	p^1	OR	p^{1*}	OR	p^2	OR	p^{2*}	OR
rs1800896								
AA	ref	1.00	0.54	1.00	ref	1.00	0.94	1.00
GG	0.86	0.91[0.32–2.54]		1.43[0.63–3.26]	0.81	0.8[0.33–2.4]		0.87[0.37–2.01]
AG	0.38	1.43[0.63–3.25]		0.91[0.33–2.54]	0.74	0.8[0.37–2.0]		0.89[0.33–2.38]
G	0.97	1.01[0.60–1.66]			0.17	1.5[0.83–2.7]		
rs1800871								
TT	ref	1.00	0.81	1.00	ref	1.00	**0.02****	1.00
TC	0.96	0.98[0.45–2.11]		0.99[0.46–2.1]	**0.02****	**2.45[1.11–5.4]**		**2.46[1.11–5.43]**
CC	0.83	1.72[0.32–9.10]		1.72[0.33–9.1]	0.06	5.06 [0.8–36.3]		**5.21 [1.12–24.2]**
T	0.71	1.11 [0.61–2.05]			**0.005****	**2.23 [1.25–3.9]**		
rs1800872								
CC	ref	1.00	0.96	1.00	ref	1.00	**0.03****	1.00
CA	0.97	0.98[0.45–2.12]		0.99[0.46–2.13]	**0.02****	**2.39[1.09–5.26]**		**2.40[1.09–5.27]**
AA	0.89	1.25[0.26–5.97]		1.25[0.26–5.97]	0.13	3.60[0.68–16.1]		**3.68[0.88–15.2]**
C	0.87	1.05[0.57–1.92]			**0.01****	**2.07[1.16–3.67]**		
rs1554286								
CC	ref	1.00	0.49	1.00	ref	1.00	0.07	1.00
CT	0.41	1.36[0.64–2.88]		1.36[0.64–2.88]	**0.02****	**2.40[1.10–5.24]**		2.41[1.11–5.24]
TT	0.84	1.78[0.17–18.4]		0.45[0.05–4.20]	0.64	2.05[0.42–10.0]		2.05[0.42–10.0]
C	0.83	1.06[0.57–1.97]			**0.04****	**1.82[1.01–3.28]**		
rs1878672								
CC	ref	1.00	0.77	1.00	ref	1.00	**0.026****	1.00
CA	0.60	0.79[0.33–1.88]		0.80[0.34–1.88]	0.07	0.43[0.16–1.10]		**0.43[0.17–1.10]**
AA	0.49	0.69[0.23–2.01]		0.69[0.24–2.00]	**0.03****	**0.16[0.01–0.86]**		**0.16[0.03–0.80]**
C	0.46	0.81[0.47–1.40]			**0.006****	**0.32[0.13–0.74]**		
rs1518111								
GG	ref	1.00	0.51	1.00	ref	1.00	0.15	1.00
GA	0.83	1.08[0.50–2.32]		1.21[0.57–2.56]	0.12	0.82[0.84–3.96]		1.38[0.84–3.96]
AA	0.45	2.43[0.51–11.8]		2.46[0.51–11.8]	0.25	3.36[0.68–24.7]		3.37[0.69–16.4]
G	0.31	1.35[0.76–2.42]				**0.05****	**1.75[0.98–3.13]**	

SNP: single nucleotide polymorphisms; OR: Odds Ratio; CI: Confidence Interval; **: significant association p < 0.05; p^1 value: chi-square test between (Chronic patients (G1) and cirrhosis group (G2)); p^{1*}: one way anova between G1vsG2; p^2 value: chi-square test between (Chronic patients (G1) and HCC group (G3)); p^{2*}: one way anova between G1vsG3.
Bold values indicate p < 0.05.

5.21 (1.09–5.27)], and rs1800871 minor (T) allele frequency was significantly between CHC and HCC patients [*P* = 0.005, OR (95%CI) = 2.23 (1.25–3.9)]. In addition, rs1800872 C/C genotype [*P* = 0.03, OR (95%CI) = 2.40 (1.09–5.27)] and minor (C) allele [*P* = 0.01, OR (95% CI) = 2.07 (1.16–3.67)] were associated with protection against HCV infection. Positive association was identified in the association

of rs1518111 [$P = 0.05$, OR (95%CI) = 1.75 (0.98–3.13)] and rs1554286 [$P = 0.04$, OR (95%CI) = 1.82(1.01–3.28)] minor alleles, but not at the genotype level.

On the other hand, rs1878672 C/C genotype [$P = 0.026$, OR (95% CI) = 0.16 (0.03–0.80)] and minor allele [$P = 0.006$, OR (95%CI) = 0.32(0.13–0.74)] appeared to be protective of disease progression and development of HCC. Interestingly, no association between patients with chronic infection and patients with cirrhosis and the distribution of the six tested SNPs was seen.

3.3. IL-6 polymorphisms and HCV outcomes

IL-6 rs1800796 G/G genotype was identified as non-favourable for chronic HCV infection progression to cirrhosis [$P = 0.023$, OR (95%CI) = 3.44 (1.30–9.1)] and HCC [$P = 0.019$, OR (95%CI) = 3.9 (1.47–10.37)]. Similarly, rs1474358 minor (C) allele, which was shown to be non-favourable for progression of chronic HCV infection to cirrhosis [$P = 0.013$, OR (95%CI) = 2.45 (1.18–5.04)], However, rs1474358 G/G genotype had a favourable outcome for HCC development [$P = 0.05$, OR (95%CI) = 0.53(0.22–1.30)]. Compared to patients with HCC, significantly higher rs1800797 minor (A) allele frequency was seen in patients with HCV (20.4% vs. 41%), which confirm the negative association of rs1800797 in aggravation of the HCV infection [$P = 0.04$, OR (95%CI) = 0.44 (0.20–0.97)] (Table 2).

3.4. Correlation between IL-10, IL-6, Alpha-fetoprotein and HCC installation

High AFP serum levels have been shown to be correlated to tumor size and HCC aggravation [26,27]. Patients were stratified according to favourable and non-favourable genotypes found associated with/against disease aggravation for *IL-10* and *IL-6* SNPs. The frequency of *IL-10* rs1800872 G/G and A/A genotype, and mean AFP

Table 2
IL-6 polymorphisms and HCV infection outcomes.

SNPs	p^1	OR	p^{1*}	OR	p^2	OR	p^{2*}	OR
rs1880242								
TT	ref	1.00	0.27	1.00	ref	1.00	0.23	1.00
TG	0.13	0.55[0.25–1.20]		0.56[0.26–1.19]	0.08	0.5[0.22–1.1]		0.50[0.23–1.11]
GG	0.88	1.10[0.30–3.97]		1.10[0.30–3.98]	0.88	0.6[0.15–2.9]		0.69[0.16–2.99]
G	0.45	0.80[0.46–1.41]			0.17	1.5[0.83–2.7]		
rs1474347								
TT	ref	1.00	0.43	1.00	ref	1.00	0.6	1.00
TG	0.25	0.63[0.28–1.40]		0.63[0.28–1.4]	0.37	0.69[0.3–1.55]		0.69[0.31–1.56]
GG	0.97	1.47[0.28–7.72]		1.47[0.28–7.7]	0.99	0.53[0.05–5.4]		0.54[0.05–5.40]
G	0.60	1.18[0.62–2.29]			0.78	1.09[0.57–2.1]		
rs2069840								
CC	ref	1.00	0.96	1.00	ref	1.00	0.94	1.00
CG	0.77	1.11[0.53–2.34]		1.12[0.53–2.34]	0.74	1.13[0.52–2.44]		1.14[0.53–2.45]
GG	0.96	1.03[0.22–4.70]		1.04[0.23–4.70]	0.83	1.17[0.25–5.35]		1.17[0.26–5.35]
G	0.94	0.93[0.52–1.66]			0.73	0.90[0.50–1.62]		
rs1800797								
GG	ref	1.00	0.13	1.00	ref	1.00	0.06	1.00
GA	0.43	1.53[0.52–4.4]		0.56[0.24–1.29]	0.09	0.48[0.20–1.15]		0.49[0.20–1.16]
AA	0.84	1.78[0.17–18.4]		2.50[0.56–11.2]	0.15	0.00		0.00[0.00–0.00]
A	0.99	1.00[0.53–1.86]			**0.04****	**0.44[0.20–0.97]**		
rs1800796								
GG	ref	1.00	**0.023***	1.00	ref	1.00	**0.019****	1.00
GC	**0.0009***	**3.44[1.30–9.08]**		**3.44 [1.30–9.1]**	**0.004****	**3.9[1.46–10.37]**		**3.9[1.47–10.37]**
CC	0.52	4.23[0.37–48.3]		**4.24[0.37–48.3]**	0.88	2.4[0.14–39.64]		**2.4[0.15–39.64]**
C	**0.004***	**0.31[0.13–0.71]**			**0.006****	**0.32[0.13–0.74]**		
rs2069845								
GG	ref	1.00	0.91	1.00	ref	1.00	0.32	1.00
GA	0.91	1.04[0.48–2.22]		1.04[0.49–2.23]	0.14	0.53[0.22–1.25]		0.54[0.23–1.25]
AA	0.65	1.37[0.33–5.53]		1.37[0.34–5.53]	0.55	0.60[0.10–3.28]		0.60[0.11–3.28]
A	0.7	0.89[0.50–1.60]				0.16	1.61[0.82–3.18]	
rs2069827								
GG	ref	1.00	0.58	1.00	ref	1.00	0.70	1.00
GT	0.87	0.91[0.31–2.66]		0.92 [0.32–2.6]	0.47	0.64[0.19–2.15]		0.64[0.19–2.16]
TT	0.66	3.36[0.29–38.2]		3.37 [0.30–38.2]	0.68	1.76[0.10–3.28]		1.77[0.11–29.1]
T	0.54	0.76[0.32–1.82]				0.73	1.19[0.43–3.25]	
rs1474348								
GG	ref	1.00	0.84	1.00	ref	1.00	**0.05****	1.00
GC	0.79	0.89[0.39–2.03]		0.90[0.40–2.03]	0.15	0.52[0.21–1.28]		**0.53[0.22–1.20]**
CC	0.64	1.28[0.43–3.82]		1.29[0.43–3.83]	**0.05****	**0.15[0.02–1.29]**		**0.16[0.02–1.30]**
C	0.77	0.92[0.52–1.62]			**0.013****	**2.45[1.18–5.04]**		
rs1800795								
GG	ref	1.00	0.94	1.00	ref	1.00	0.42	1.00
GC	0.84	0.92[0.41–2.07]		0.92[0.41–2.07]	0.28	0.62[0.25–1.5]		0.62[0.26–1.50]
CC	0.88	1.22[0.25–5.83]		1.23[0.26–5.83]	0.4	0.39[0.04–3.7]		0.40[0.04–3.70]
C	0.96	0.98[0.52–1.85]			0.18	1.65[0.78–3.48]		

SNP: single nucleotide polymorphisms; OR: Odds Ratio; CI: Confidence Interval; **: significant association $p < 0.05$; p^1 value: chi-square test between (Chronic patients (G1) and cirrhosis group (G2)); p^{1*}: one way anova between G1vsG2; p^2 value: chi-square test between (Chronic patients (G1) and HCC group (G3)); p^{2*}: one way anova between G1vsG3.
Bold values indicate $p < 0.05$.

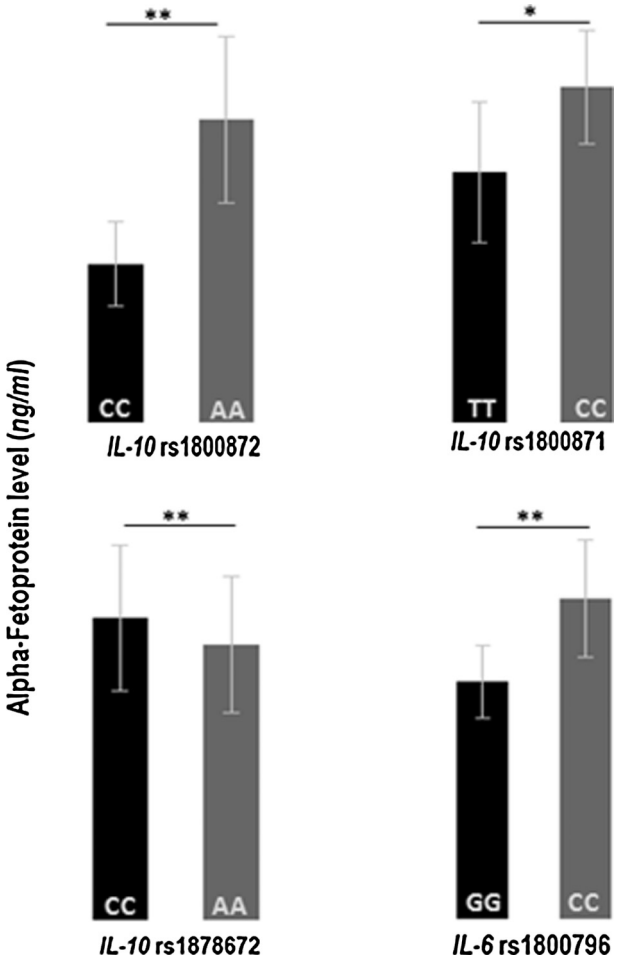

Fig. 2. Level of Alpha fetoprotein in patients with HCC stratified by genotype.

level was statistically different ($P = 0.03$), as well as *IL-10* rs1878672 C/C and A/A genotypes ($P = 0.01$). AFP levels were more pronounced in *IL-6* rs1800796 C/C compared to G/G genotype carriers ($P = 0.002$). AFP levels among patients with HCC and carriers of *IL-10* rs1800871 T/T and C/C genotypes were not significantly different ($P = 0.07$). Interestingly, the AFP levels were in accordance to what has been found in IL-10 and IL-6 genotype analysis (Fig. 2).

4. Discussion

Significant differences were seen among patient groups in terms of age, AST, ALT, AFP, Actitest and Fibrotest stages, with patients in cirrhosis and HCC stage presenting with severe fibrosis, and high necro-inflammatory activity. An interesting finding was the cross-regulation of cytokine expression associated with increased necrosis, consistent with inflammation accompanying liver fibrosis [28,29], which correlated with elevated IL-6 and IL-17, more than IL-10, levels [29], suggesting that IL-10 regulates inflammation-induced fibrosis and carcinogenesis. Thus, the severity of fibrosis, and high necro-inflammatory activity in HCC patients is attributed to low IL-10 production, and consequently excessive activity of hepatic tissue [30]. This suggests that control of IL-10 production in patients with moderate/severe fibrosis significantly slows down the HCC development, and thus the quality of life.

IL-10 influences inflammatory responses, and acts as anti-inflammatory and anti-angiogenic cytokine, hence facilitating tumor growth directly. It also acts indirectly by inhibiting anti-tumor immunity [31]. Previous studies showed that IL-10 promoter variants influence IL-10 levels [32–34], highlighted by the finding that rs1800871 (−819C/T) and rs1800872 (−592C/A) SNPs are linked with low IL-10 expression [35,36]. Significant association was seen between HCV outcomes and rs1800871, rs1800872, and rs18786723 *IL-10* SNPs. Negative association between HCC and rs1800871T/T and rs1800872C/C genotypes was seen; the latter acting as a positive factor for aggravation of HCV infection, hence HCC development. Since rs1800871 T/T and rs1800872C/C genotypes are low IL-10 producer genotypes [38], this suggests that patients with high IL-10 levels are likely to develop severe forms of chronic liver disease.

Our results are in accord with earlier studies, which documented higher serum IL-10 levels in patients with severe chronic liver disease than in controls [38]. It is likely that high IL-10 levels was due to its over production by tumor cells, which in turn contributes to the state of tumor-induced immunosuppression which facilitates tumor escape from host immune surveillance, and thus enhances metastasis in patients with HCC [39]. This notion was supported by an Egyptian study [40], which found that basal IL-10 was undetectable in chronic patients with HCV infection, and in HCV-infected patients with HCC. In addition, an association between high levels of IL-10 and AFP levels was noted when patients were analyzed according to rs1800871, rs1800872 and rs1878672 genotypes. Recent studies reported significant relation between elevated AFP and HCC on one hand, and the number of nodules and advanced stages of HCC on the other [41,42]. This suggests that high IL-10 levels correlated with high AFP levels may indicate detachment of HCC cells from tumor cells into the circulation, thus metastatic stage represents a severe stage when controlling the carcinoma becomes increasingly difficult.

Chronic inflammation ensues when inflammatory cells are recruited to the affected (inflamed) site, coupled with induction of anti-apoptotic mechanisms [43]. IL-6 plays a key role in this process, owing to its dual capacity as pro- and anti-inflammatory cytokine, which in turn sustains the cellular growth and anti-apoptotic activities accompanying chronic inflammation [44]. Several functional and non-functional polymorphic variants were described throughout *IL-6* gene, some of which linked with severe chronic HCV infection and HCC [45]. In our study, rs1800796 *IL-6* variant, linked with high *IL-6* level [46], was associated with the presence of HCC, suggesting a role for IL-6 in HCC pathogenesis. This was supported by earlier studies documenting increased serum IL-6 levels in patients with chronic liver disease, including HCC patients [47]. Reichner reported on the need for IL-6 for the spread of HCC cells beyond the liver [48], based on the findings that highly metastatic cell lines derived from HCC are likely to produce very high levels of IL-6, compared to poorly metastatic cells from the same tumor.

This correlation was true in our patients, as AFP levels were significantly higher in patients with HCC who are also high IL-6 producers. Our results are in accord with an Italian study, which documented an association between high IL-6 production and the tumor size in HCC [45]. It is noteworthy that AFP is positively correlated with HCC aggravation, as well as IL-6 level [27,41,42]. We also found that elevated IL-6 levels in HCV infected patients are associated with disease progression, which may be attributed to the capacity of IL-6 to decrease apoptosis of HCC cells, thereby conferring survival advantage for tumor cells. Moreover, IL-6 was linked with natural killer cell dysfunction, which may provide a mechanism of tumor escape from immune surveillance [49].

In conclusion, IL-10 and IL-6 are key cytokines in infection, cancer and inflammation. Their altered regulation in disease progression and aggravation is exacted at the stage of immune surveillance. It's therefore essential to establish how these potential cytokines contributes to disease development, notably cancer installation providing an opportunity to guide treatment and

improve rates of clinical cares. However, the most challenging part is to understand the difference between good and bad effects of IL-10 and IL-6 signalling pathways. Aiming an effective treatment and attack against HCC installation.

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