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TLR3 and *TLR4* SNP variants in the liver disease resulting from hepatitis B virus and hepatitis C virus infection

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ABSTRACT

Background: Chronic infection with hepatitis B (HBV) and C virus (HCV) is linked with a pro-inflammatory state, predisposing to cirrhosis and liver cancer, particularly hepatocellular carcinoma (HCC). A role for Toll-like receptor (TLR) signalling in hepatocarcinogenesis was recently documented. We hypothesised a link TLR3 and TLR4 polymorphisms and HCC, as surrogates for the significance of TLR signalling in the promotion and initiation of HCC.

Materials and methods: We recruited 174 HCV-infected patients, 100 HBV-infected patients and 360 healthy control subjects. *TLR3* (rs3775290) and *TLR4* (rs4986790) genotyping was done by PCR-restriction fragment length polymorphisms (PCR-RFLP), LFTs and AFP by standard routine techniques. Liver fibrosis was assessed clinically by the Fibrotest and Actitest.

Result: The *TLR3* rs3775290 minor T genotype was linked with increased risk of chronic HBV ($P = 0.05$) and HCV ($P = 0.031$) infection. The *TLR4* rs4986790 minor G genotype was linked with significantly increased risk for HBV/HCV chronic infection ($P < 0.001$). Subgroups analyses indicated decreased risk of HBV-related HCC in relation to *TLR3* rs3775290 CC/CT genotype ($P = 0.022$), with increased risk ascribed to the minor (T) allele ($P = 0.04$). Likewise, *TLR4* rs4985790 minor (GG) genotype was positively associated with HBV-linked HCC ($P < 0.001$). Furthermore, a link between *TLR3* TT ($P < 0.001$) and*TLR4* GG ($P = 0.04$) minor genotypes was noted in relation to increased risk of HCV-related disease.

Conclusion: *TLR3* and *TLR4* polymorphisms are promising biomarkers of liver cirrhosis and cancer associated with HBV and HCV infection.

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KEYWORDS
HBV; HCV; hepatocellular carcinoma; Toll-like receptors

Background

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. With almost one million new cases and approximately 600,000 deaths recorded per year, HCC ranks as the fifth most common cancer type worldwide, and the second leading cause of cancer-related mortality [1]. As chemotherapy and radiotherapy are often not curative, surgery remains the management of HCC in early and mid-stages. Disease recurrence is common after radical resection, and the 5-year post-surgery survival rates range from 30% to 40% [2]. However, the exact mechanism underlying the development and progression of HCC remains unknown, although hepatitis B virus (HBV) and hepatitis C virus (HCV) precipitate HCC in the absence of other oncogenic events [3], a process marked by hepatocyte proliferation and differentiation during chronic liver injury [4]. Indeed, chronic viral infections are major risk factors for cancer development [1], highlighted by the finding that 80% of HCC are linked to HBV and HCV infection [5]. Chronic viral infection brings a 10- to 100-fold the risk of HCC, compared to non-infected individuals, further underscoring the relationship between host factors and HBV/HCV infection with subsequent cancer occurrence [2]. In one study, almost two-thirds of HCC was attributed to HCV infection [6]. Accordingly, reduction in the burden of chronic HCV and HBV infection is likely to result in a significant decrease in HCC incidence.

Anti-viral immunity is associated with a complex series of immune signals, with monocyte and macrophage activation [7]. Pathogens are detected through germline-encoded (innate) immune surveillance pattern recognition receptors (PRR), which recognise conserved pathogen-associated molecular patterns (PAMP) [8]. PRR belong to family of receptors, including Toll-like receptors (TLR), which initiate specific anti-viral immune responses [8]. TLR3 is an intra-cellular receptor that recognises viral double-stranded RNA (ds-RNA), and initiates inflammatory response against DNA (HBV) and RNA (HCV) viruses [9]. TLR3 influences the chronicity of virus infection, and thus subsequent pathological changes, including liver cirrhosis and HCC, while TLR4 affects viral replication through induction of IFN α/β

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transcription [10,11], and its link with tumour severity and chronic viral infection was highlighted by the finding that HCV protein induces TLR4 expression [12].

Heterogeneity in TLR3 and TLR4 expression and levels are due to specific intronic and exonic gene variants in both genes [13]. Of these, *TLR3* rs3775290 and *TLR4* rs4986790 single nucleotide polymorphisms (SNPs) modify cancer susceptibility, and thus have been proposed as potential biomarkers of cancer risk [14,15]. We therefore hypothesised links between these SNPs and the liver disease that inevitably follows hepatitis virus infection, that is, liver cirrhosis and HCC.

Subjects and methods

We recruited 100 chronic HBV carriers and 174 chronic HCV carriers at Charles Nicolle Hospital in Tunis, Tunisia. In addition, 360 individuals who were seronegative for both HBV and HCV served as the control group (Table 1). HBV- or HCV-infected patients were sub-grouped into patients with chronic hepatitis, those with liver cirrhosis and those with HCC. Inclusion criteria were HBsAg seropositivity (HBV group), or positive anti-HCV antibodies and HCV RNA (HCV group) for ≥ 6 months, with or without persistently elevated alanine aminotransferase (ALT) levels. Individuals who did not meet these criteria or were co-infected with hepatitis D virus (HDV) and/or HIV were excluded. Additional exclusion criteria included Wilson's Disease, autoimmune hepatitis and non-alcoholic steatohepatitis in the absence of diabetes, hypertriglyceridemia and related risk factors. Clinical and laboratory evaluation included biochemical [ALT and aspartate aminotransferase (AST)] and serological tests for HBV (HBsAg, HBeAg, anti-HBeAg, anti-HBc_{total}) and HCV (anti-HCV), and histopathology of liver biopsy. HCC was diagnosed by ultrasound, computerised tomography, magnetic resonance imaging, arteriography and tumour biopsy. Patients were characterised by alpha-fetoprotein (AFP) levels, and further classified by METAVIR score [16]; F0–F2 scores indicated mild fibrosis, while F3 and F4 indicated severe fibrosis. Necro-inflammatory activity was graded on 0–3 scale according to METAVIR activity grading. LFTs and AFP were measured by standard methods in the routine pathology laboratory. The approval of the local research ethics committee was obtained, as was written informed consent from each subject.

Genomic DNA was extracted from peripheral venous blood using QIAamp® DNA Blood Mini Kit, per manufacturer's instructions (Qiagen GmbH, Hilden, Germany). *TLR4* rs4986790 and *TLR3* rs3775290 genotyping was carried out by PCR-restriction fragment length polymorphism analyses. Amplification conditions comprised initial denaturation (95°C for 5 min), followed by 35 cycles of denaturation (95°C for 45 s), annealing (55°C for 45 s) and extension (72°C for 30 s), and final extension (72°C for 7 min). PCR products were digested by *Taq I* (*TLR3* rs3775290) and *NcoI* (*TLR4* rs4986790) restriction enzymes and were visualised in 4% agarose gel electrophoresis.

Statistical analysis and graphing was performed with R and R studio program, version 3.2.3 (https://www.r-project.org/). Continuous variables were expressed as mean (SD), while categorical data were expressed as percent of total. For non-normal distribution the results was expressed on median with IQR. Independent sample *t*-test and Mann-Whitney was used for inter-group comparisons of continuous data, and χ^2 test (or Fisher's exact test for low numbers and Kruskal-Wallis for the non-normal distribution) was used in analysing categorical variables. SNPs were analysed for Hardy-Weinberg equilibrium (HWE) by χ^2 test, equilibrium established with $P > 0.05$. Association analysis was evaluated by setting homozygous major allele genotype as reference (OR = 1.00); subsequent analysis was done using one-way ANOVA. Logistic regression analysis was used to investigate the relationship among *TLR3* and *TLR4* variants, HBV or HCV outcomes, and the independent contribution

Table 1. Baseline characteristics of patients infected with HCV and HBV.

	HCV patients (n = 174)				HBV patients (n = 100)			
	Chronic infection N = 81	*Cirrhosis N = 49*	*HCC N = 44*	p^1	*Chronic infection N = 48*	*Cirrhosis N = 30*	*HCC N = 22*	p^2
Men/Women (%)	58.0/42.0	59.2/40.8	63.6/36.4	0.82	47.9/52.1	56.6/43.4	90.9/9.1	0.0022
Age (years)	43.3 (13.9)	47.8 (17.3)	53.8 (14.3)	0.001	47.3 (15.9)	57.4 (15.2)	56.6 (15.3)	0.01
Actitest stage (%)								
A0-A1	65 (80.2)	30 (61.2)	11 [25]	0.05	32 (66.7)	1 (3.3)	1 (4.5)	<0.001
A2-A3	16 (19.8)	19 (38.7)	33 (75)		16 (33.3)	29 (96.7)	21 (95.5)	
Fibrotest stage (%)								
F0-F1-F2	49 (60.4)	13 (26.5)	14 (31.8)	<0.001	45 (93.8)	11 (36.7)	1 (4.5)	<0.001
F3-F4	32 (39.6)	36 (73.5)	30 (68.2)		3 (6.2)	19 (63.3)	21 (95.5)	
ALT (IU/mL)	70 (40–87)	85 (45–102)	121 (64–148)	0.02	41 (19–60)	58 (49–69)	55 (29–68)	0.872
AST (IU/mL)	68 (46–96)	87 (56–97)	103 (51–104)	0.042	31 (15–46)	60 (24–74)	67 (49–76)	0.03
AFP (ng/mL)	4.2 (2.2–6.0)	8.6 (5.6–14.1)	128 (33.3–174.3)	<0.001	3.4 (1.9–6.0)	8.2 (3.9–13.4)	62.7 (11.7–150.0)	<0.001

AST: aspartate aminotransferase; ALT: alanine aminotransferase; Data mean (SD), median (IQR) or *n* (%).
p^1: between patients infected with HCV; p^2: between patient infected by HBV; HCC: Hepato-cellular carcinoma.

of key covariates (AFP levels); $P < 0.05$ was considered statistically significant.

Results

Demographic, laboratory and clinical characteristics of participants are summarised in Table 1. There was no difference in the sex ratios of the HCV patients, but age increased with liver disease stage. There was also an age difference in the HBV patients, and there were more men with HCC. The differences in Actitest were marginally significant in the HCV group, but in the HBV group, these differences were significant. There were significant differences in Fibrotest results in both groups. AST and ALT differed between HCV patients, but in the HVB patients only the AST levels varied. In both groups, AFP was increased in cirrhosis and increased further in those with HCC.

Table 2 shows the genotype and allele distributions of TLR3 rs3775290 and TLR4 rs4986790 among patients and control subjects: genotype distributions were in HWE ($P = 0.07$ and $P = 0.63$, respectively). Significant differences in the distribution of rs3775290 alleles and genotypes was found between all 174 hepatitis C patients and healthy controls ($P < 0.001$), which remained significant after controlling for key covariates ($P < 0.001$). Increased risk (almost 1.5-fold) for rs3775290 minor allele-carriers (T) was associated with HCV infection. A marginal association was seen between rs3775290 minor allele (T) in the 100 patients with HBV infection, and almost a twofold risk of HBV infection in rs3775290 minor allele carrying patients compared to healthy controls. No significant link was found between *TLR3* rs3775290 major allele genotype and HBV infection. The *TLR4* rs4986790 homozygous major allele genotype frequency (AA) was lower in HCV-infected patients than in controls. In contrast, rs4986790 minor G allele was more frequent among HCV-infected patients. Indeed, rs4986790 minor G allele carrying patients were at almost six times higher risk of HCV. The homozygous major allele (*AA*) genotype frequency was higher in controls than HBV patients. In addition, the minor allele (*G*)-carrying patients were at 11-fold higher risk of HBV infection.

Table 3 shows links between *TLR3* and *TLR4* variants and patients with an uncomplicated viral infection, those with liver cirrhosis, and those with HCC. In the HBV-infected patients, there was no difference in *TLR3* rs3775290 genotypes or alleles linked to liver cirrhosis, but patients with HCC had a different frequency of genotypes. Both homozygous major (C/C) and heterozygote (C/T) *TLR3* rs3775290 genotypes were more frequent among cirrhotic patients, compared to HCC

Table 2. Distribution of TLR3 and TLR4 SNPs alleles in controls, HCV- and HBV-infected patients.

SNPs	Controls $N = 360$	HCV $N = 174$	p^1	OR (95% CI)	HBV $N = 100$	p^2	OR (95% CI)
TLR3 rs3775290							
CC	43.6	44.3	0.006	1.00 (Ref)	36.0	0.379	1.00 (Ref)
CT	41.4	29.3		1.47 (1.00–2.25)	37.0		0.92 (0.55–1.54)
TT	15.0	26.4		0.64 (0.36–0.93)	27.0		0.46 (0.25–0.82)
T	35.6	41.1	0.031	1.46 (1.05–2.03)	45.5	0.05	1.79 (1.08–2.95)
TLR4 rs4986790							
AA	70.3	27.0	<0.01	1.00 (Ref)	26.0	<0.01	1.00 (Ref)
AG	21.4	29.9		0.47 (0.30–0.74)	30.0		0.36 (0.20–0.67)
GG	8.3	43.1		0.22 (0.13–0.37)	44.0		0.11 (0.06–0.21)
G	19.02	41.9	<0.001	5.96 (4.09–8.74)	95.0	<0.001	11.3 (6.67–19.6)

p^1: chi-square test between controls and HCV-infected patients, p^2: chi-square test between controls and HBV-infected patients.

Table 3. Distribution of TLR3, TLR4 SNPs and HBV/HCV outcomes.

Group	SNPs	Infection alone	Liver cirrhosis	p^{a1}	OR (95% CI)	HCC	p^{a2}	OR (95% CI)
HBV patients	TLR3 rs3775290							
	CC	25.0	46.7	0.235	1.00 (Ref)	45.5	0.022	1.00 (Ref)
	CT	39.6	43.3		0.59 (0.21–1.67)	22.7		0.08 (0.01–0.72)
	TT	35.4	10.0		0.30 (0.04–0.64)	31.8		0.49 (0.13–1.00)
	T	52.0	40.2	0.07	1.77 (1.02–3.90)	36.7	0.04	1.87 (1.00–4.34)
	TLR4 rs4986790							
	GG	44.9	66.7	0.006	1.00 (Ref)	60.0	0.0011	1.00 (Ref)
	AG	38.8	23.3		3.36 (1.40–8.04)	33.4		5.27 (0.51–5.44)
	AA	16.3	10.0		3.37 (1.32–8.57)	6.6		39.5 (3.3–40.73)
	G	35.7	78.4	<0.001	1.69 (0.99–4.53)	76.6	<0.001	5.91 (2.40–16.9)
HCV patients	TLR3 rs3775290							
	CC	9.90	36.7	0.005	1.00 (Ref)	50.0	<0.001	1.00 (Ref)
	CT	29.6	30.6		1.71 (0.73–3.95)	27.3		2.83 (1.03–7.73)
	TT	60.5	32.7		5.44 (1.99–14.8)	22.7		11.52 (3.9–33.9)
	T	24.7	47.9	0.02	1.04 (1.01–1.08)	63.3	<0.001	1.07 (1.04–1.11)
	TLR4 rs4986790							
	GG	18.5	28.6	0.006	1.00 (Ref)	38.6	0.04	1.00 (Ref)
	AG	23.5	40.8		3.36 (1.40–8.04)	29.5		2.06 (0.76–5.62)
	AA	58.0	30.6		3.37 (1.32–8.57)	31.8		3.31 (1.25–8.75)
	G	30.2	51.0	0.08	1.03 (0.99–1.07)	53.4	0.06	1.02 (0.99–1.06)

a: *P* values adjusted for age and sex, by stepwise regression analysis. p^1: chi-square test between patients with chronic infection and patients with cirrhosis. p^2: chi-square test between LC patients and patients with HCC.

patients. Moreover, carriage of rs3775290 (T) minor allele was associated with twofold higher risk of HCC development after cirrhosis establishment. Significant difference in *TLR4* rs4986790 homozygous minor allele genotype (GG) frequencies was seen between patient's liver disease and those with cirrhosis, and carriage of rs4986790 G/G genotype was associated with over a threefold higher risk of cirrhosis. Homozygous minor allele genotype constituted a risk factor of HCC, and G/G genotype carrying patient were at over a fivefold higher risk of HCC. TLR4 rs4986790 minor (G) allele was also more frequent among cirrhotic patients compared to infected-alone patients. Compared to infected-only patients, a significantly higher rs4986790 minor allele (G) frequency was seen in HCC patients.

The *TLR3* rs3775290 homozygous minor allele genotype (T/T) was progressively lower in cirrhotic patients and HCC patients. Compared to patients with an uncomplicated HCV infection, significantly higher allele frequency in *TLR3* rs3775290 was seen in cirrhotic patients. The *TLR3* rs3775290 minor T allele also constituted a risk factor for the presence of HCC. The *TLR4* rs4986790 homozygous minor allele genotype (G/G) was positively associated with over a threefold risk of liver cirrhosis, However, the association between *TLR4* rs4986790 and HCV outcomes was lost after adjusting for key covariates.

Links between *TLR3* and *TLR4* variants and AFP are as follows. Figure 1(a,b) show analysis of HCV-infected patients. Classified by TLR3 rs3775290 TT/non-TT genotype, the AFP difference was $P = 0.05$ in the cases of cirrhosis, and $P = 0.058$ in patients with HCC. However, AFP was higher in the TLR4 rs4986790 GG genotype cirrhosis ($P = 0.04$) and HCC ($P = 0.023$) patients. Figure 1 (c,d) show AFP in patients with HBV. Classification by TLR3 rs3775290 TT status found a borderline difference in patients with a simple chronic infection ($P = 0.05$), and higher levels in cirrhosis ($P = 0.044$) and in HCC ($P = 0.016$). Figure 1(d) shows that the *TLR4* rs4986790 homozygous minor genotype (G/G) was linked with elevated AFP levels in cirrhosis ($P = 0.04$) and HCC ($P = 0.0049$) patients.

Discussion

In both HBV and HCV cohorts, increased age and male sex were linked to disease severity (uncomplicated infection < cirrhosis < HCC), as reflects the general course of these infections and is expected [1,18,19], as is the deterioration of clinical scores Actitest and fibrotest [20]. While significant differences in AST and ALT levels in disease stages were noted, these were higher in HCV infection than in HBV infection [21]. Similarly, AFP levels closely followed the three disease stages. It remains to be seen which of these

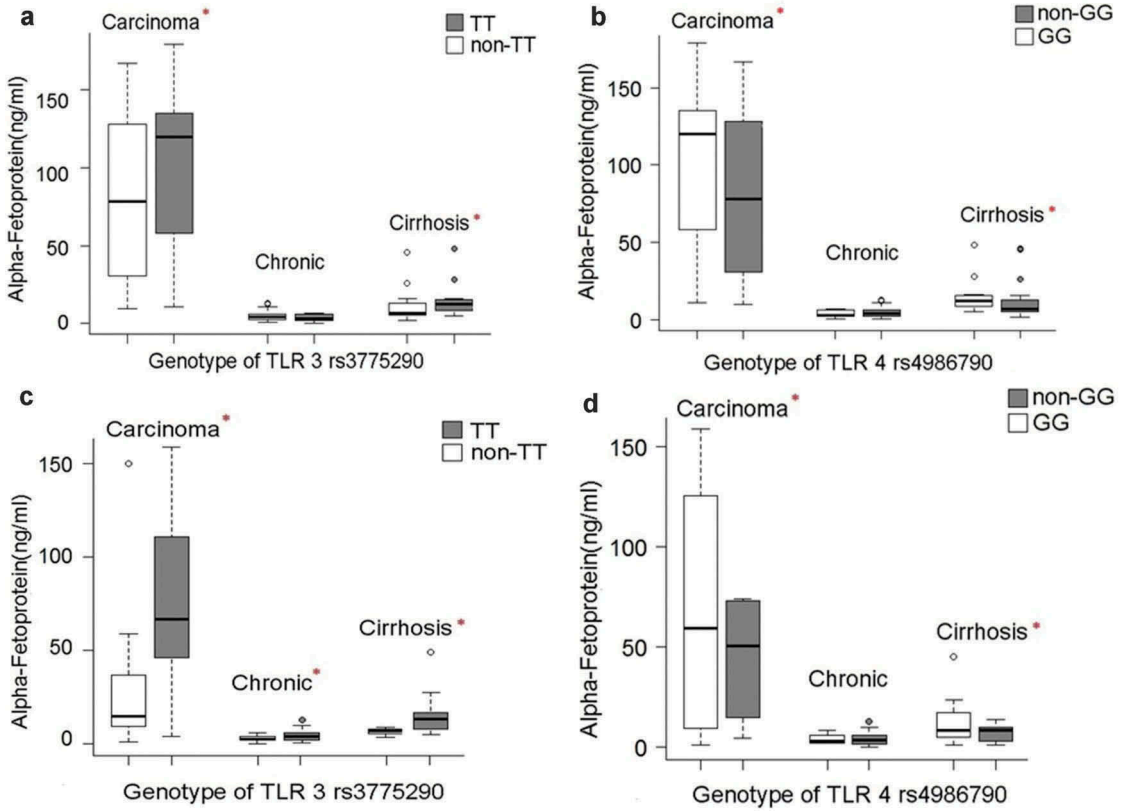

Figure 1. (a) Levels of alpha fetoprotein (AFP) in patients with HCV infection stratified by TLR3 genotype according to disease stage (chronic infection, cirrhosis, HCC). (b) Levels of alpha fetoprotein (AFP) in patients with HCV infection stratified by TLR4 genotype according to disease stage (chronic infection, cirrhosis, HCC). (c) Levels of alpha fetoprotein (AFP) in patients with HCB infection stratified by TLR3 genotype according to disease stage (chronic infection, cirrhosis, HCC). (d) Levels of alpha fetoprotein (AFP) in patients with HCB infection stratified by TLR4 genotype according to disease stage (chronic infection, cirrhosis, HCC).

tests constitute reliable surrogates for predicting and disease severity.

Persistence of HBeAg seronegativity (>6 months) in HBV-infected patients results from mutations in pre-core and basal core promoter regions of HBV DNA, which results in attenuation of HBeAg synthesis, without affecting HBV replication [17]. As the patients included in HBV group were HBeAg-negative, hence infected with mutant HBV genotype. HBV group was stratified into carriers of HBV wild-type and mutant genotypes, and AFP levels were determined according to favourable and non-favourable *TLR3* and *TLR4* genotype (see above). *TLR3* rs3775290 homozygous minor allele genotype remains a favourable factor for HBV infection aggravation among wild-type and mutant HBV genotypes. However, *TLR4* rs4986790 homozygous minor allele genotype constituted a risk factor in patient carriers of HBV wild-type, but not mutant genotypes.

Effective detection and control of viral replication depends on viral and host immunity. TLR3/4 are key components of innate immunity, involved in detection of viral infection, thus triggering anti-viral immunity by pathogen selection pressure [22]. The high rates of *TLR* genetic SNPs such as *TLR3* rs3775290 and *TLR4* rs4986790 may affect gene function [22]. TLR3 plays a crucial role in HBV and HCV infection, prompting the speculation that TLR3 is directly involved in host defence against virus infection by its ds-RNA response [9]. Insofar as specific gene variants are implicated in modification of gene expression, *TLR3* rs3775290 may be linked with severe outcomes of HBV and HCV infection. Our data support this notion, showing that *TLR3* rs3775290 minor (T) allele is linked with increasing severe disease in HBV and HCV infection, perhaps by blunting TLR3 signalling in response to viral infection in dominant-negative manner. This suggests that this variant may hinder the interaction with HBV DNA or HCV ds-RNA, and thus reduce TLR3-mediated signalling [23–25], which modifies cancer susceptibility. Our results showed significant differences in distribution of alternate alleles in earlier stages of infection, and advanced severe liver disease, perhaps linked to the maturation of dendritic cells and activation of NK cells and cytotoxic T lymphocytes [26,27]. Accordingly, we speculate that *TLR3* rs3775290 contributes to HCC by regulating its expression, and so a defective TLR3 pathway. TLR4 is the LPS-recognising receptor and plays a role in disease and anti-viral immunity. In our hands *TLR4* rs4986790 SNPs were linked with liver disease, with a higher frequency of G/G genotype seen among HBV (43.1%) and HCV (44%) infected patients, compared to healthy controls (8.3%). This prompts the speculation that *TLR4* rs4986790 minor allele is involved in persistence of viral infection, conferring resistance to anti-viral immunity and suggests that those with *TLR4* rs4986790 had blunted response to LPS, as noted elsewhere [28]. The role of TLR4 in controlling HCV and HBV infection is in intra-cellular signalling pathways in hepatocytes, and in the reduction of HBV replication in an IFN-independent manner [29]. Consequently, *TLR4* rs4986790 may be associated with a perturbation of TLR4 signalling, down-regulation of the immune system performance resulting to insufficient anti-viral response.

To further illustrate the functional aspect of *TLR4* rs4986790, we analysed its distribution in simple infection, liver cirrhosis and HCC. *TLR4* rs4986790 homozygous minor allele genotype remains a risk factor for cirrhosis and HCC in HBV- and HCV-infected patients, prompting the hypothesis that *TLR4* variants modulate efficient anti-viral responses, possibly by reduced LPS responsiveness of the mutant TLR4 protein, which is mandatory for the TLR4 ectodomain to recognise LPS [30], so enhancing viral escape from immune surveillance and detection in the face of dampened anti-viral immunity [25]. Previous studies documented that mutations in molecules involved in TLR4 signalling are associated with lymphoma [31,32]. Our AFP data in respect of *TLR3*/*TLR4* favourable or non-favourable genotypes were similar in patients infected by mutant HBV genotype, regardless if they were carriers of TLR3 favourable or non-favourable genotypes. However, with the exception of $P < 0.005$ in HCC in the *TLR4* rs4986790 homozygous minor G/G genotype (G/G), differences were small. We speculate that TLR4 immune modulatory effect is dictated by HBV genotype, in accord with other studies showing that HBeAg down-regulates immune surveillance to HBV and HBV infected cells by inhibiting PRR expression and IFN signalling [23,33].

The carriage of *TLR4* Asp299Gly variant results in poor responsiveness in TLR signalling, which in turn facilitates HBV escape from immune surveillance, and consequently persistence of HBV, as highlighted by the differences of AFP levels. Therefore, it is notable that the mutant HBV genotype runs a relatively aggressive course, with frequent development of cirrhosis and HCC [34–36]. While our data indicate that *TLR3* rs3775290 and *TLR4* rs4986790 negatively impact HBV and HCV anti-viral immunity, there are instances where the *TLR3* and *TLR4* variants may enhance immunity. This is explained in part by the early control imparted by overwhelming the viral infection, which requires fast functional anti-viral innate immunity. We suggest that heightened inflammatory responses to environmental stimuli may confer greater protection from viral infection. Based on TLR3/4 data, hepatologists can more carefully monitor viral kinetics to improve the chances of success viral clearance as it is the case of IL28B biomarkers which were effective tools for prognosis of treatment response and viral clearance [37].

Our study is limited by the relatively small sample size, partially attributable to the inclusion criteria, and

the unknown duration of the viral infections, which is likely to influence rates of cirrhosis and HCC. Accordingly, having taken a single sample, we cannot say if these SNPS are linked directly to actual disease progression over time. Nevertheless, our data offer new and useful tools to study the natural history of these infections, and so may help develop new anti-virals and vaccines. As these variants may modulate the immunity response pathways, the result of our study could lead to a personalised approach for HBV/HCV infection-management to achieve safe and effective viral clearance among patients with chronic hepatitis B/C.

This work represents an advance in biomedical science because it shows that TLR 3 and 4 SNPs could be a biomarkers to determine the course of HBV and HCV infection, and would be of use to predict the efficiency of immune system during a viral infection.

Summary table

What is known about this subject:

- Specific HBV and HCV proteins may enhance the occurrence of HCC in the absence of other oncogenic factors;
- Chronic viral infection may increase from 10- to 100-fold the risk of HCC;
- Genetic variants in *TLRs* may modulate the immunity response and are involved in HCC development by contributing to HBV and HCV clearance.

What this paper adds::

- The minor allele of TLR3 is linked to HBV and HCV infection and risk of HCC;
- The minor variant of TLR4 promotes HCV infection, and the development of cirrhosis and HCC.

Disclosure statement

No potential conflict of interest was reported by the authors.

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