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Can NLRP3 gene polymorphism in Egyptian chronic hepatitis C patients affect the degree of liver fibrosis?

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ABSTRACT

Background: The cytokines genes polymorphisms are important determinants for the outcome of HCV infection and degree of liver fibrosis and inflammation. Recently, it has been revealed that hepatocytes and hepatic macrophages produce mature IL-1β through the NLRP3 (NOD-like receptor, pyrin domain containing 3) inflammasome assembly which represents a link between hepatitis C virus infection and liver inflammation. In addition, NLRP3 inflammasome has emerged as a cytoplasmic sensor of HCV participating in eliciting the innate immune response against HCV.
Objective: To explore the association of a genetic variation within 3'UTR NLRP3 gene with the degree of liver fibrosis and /or liver inflammation. Moreover, to investigate possible relation between fibrosis and the HCV load quantified at the diagnosis of Egyptian HCV patients.
Methods: We studied the distribution of genotypes and alleles of one NLRP3 SNP (rs10754558) in one hundred-forty seven chronic HCV patients using Taq Man predesigned SNP genotyping assay.
Results: The genotype distribution and allele frequencies of NLRP3 (rs10754558) polymorphism did not differ significantly neither with the degree of liver fibrosis nor with the degree of liver inflammation or the baseline HCV quantity.
Conclusions: NLRP3 (rs10754558) polymorphism was not associated neither with the degree of liver fibrosis nor with the degree of liver inflammation and amplitude of baseline HCV load measured during diagnosis of Egyptian patients chronically infected with HCV genotype 4a. Importantly, the pivotal role played by the NLRP3 in the immune response against HCV infection requires further studies for other polymorphisms within NLRP3 gene to unravel their role in HCV infection.

Keywords: Hepatitis C virus, NLRP3 Inflammsomes, liver fibrosis, liver inflammation

Introduction

Hepatitis C virus (HCV) represents one of the global public health problems. Its prevalence has expanded intensely over the last 15 years to reach more than 185 million infections worldwide [1]. Egypt has the highest HCV prevalence worldwide, with an predictable overall prevalence of 21.9% between adults [2].The major burden from HCV infection comes from sequelae from chronic infection [3]. About 75% of the infected patients become chronically infected and at risk of developing cirrhosis and liver cancer [3]. An important factor in the pathogenesis of chronic HCV disease is liver damage sustained by the development of inflammation and tissue fibrosis. Scoring of these two criteria is used as a measure of disease state and progression [4-5].The prognosis of HCV infection is the result of complex interaction between viral virulence factors and host's response mechanism which may vary from spontaneous recovery to asymptomatic patient, and may progress to liver fibrosis. Stimulation and release of proinflammatory cytokines, IL-1β and IL-18, is an essential step for the activation of an effective innate host defense, and subsequently for the modulation of adaptive immune responses [7]. Notably; these cytokines have found to play a dual role in HCV viral infection. In acute infection, these cytokines have an antiviral effect assisting in viral clearance. Besides, IL-1β has been shown to hinder replication of HCV. This means that they may

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influence the baseline viral load of HCV[8-10].On the other hand, IL-1β is a master cytokine in inflammation and it regulates diverse cellular processes in chronic HCV infection. It induces potent inflammatory molecules such as cox-2, nitric oxide and TNF-α [11]. The persistent induction of IL-1β by hepatic macrophages in chronic hepatitis C patients would then help to recruit immune cells to the liver causing liver inflammation. Most importantly, persistent liver inflammation during HCV infection is thought to serve as a platform for progressive liver injury,liver cirrhosis and liver cancer[12-13]. Interleukin-1β is synthesized as an inactive precursor that is processed into the biologically active form through recruitment of the inflammasome. The inflammasome is multi-protein complex consisting mainly of a Nod like receptor (NLR), adaptor molecule (ASC) and procaspase-1. Upon assembly of the inflammasome, caspase-1 is activated which in turn cleaves pro-IL-1β and pro-IL-18 into their mature form [14]. Numerous studies have provided evidences on the role of NLRP3 and ASC in inflammatory processes associated with chronic HCV[15-16]. Recently, it has been revealed that hepatocytes and hepatic macrophages produce mature IL-1β through the NLRP3 inflammasome assembly which represents a link between hepatitis C virus infection and liver inflammation [15][16]**.**In addition, the genetic factors influence progression of fibrosis. Several genetic polymorphisms were found to be associated with progression of fibrosis [17]**.** Transforming growth factor-b1 [18], complement factor-5 [19]**,** angiotensinogen [20], monocyte chemotactic protein-1 [21] and microsomal epoxide hydrolase genes [22] and Toll-like receptor 7 [23] are among the genes which have polymorphisms that were associated with the fibrosis degrees. Although, several single nucleotide polymorphisms (SNPs) in the pro-inflammatory cytokines have been extensively studied, the effect of the polymorphisms in the inflammasome genes on HCV outcome is unexplored yet. Thus the objective of the current study was to investigate the role of a genetic variation within NLRP3 gene (rs10754558) in the degree of liver fibrosis and inflammation Egyptian patients chronically infected with HCV. Besides, determination of its role in the extent of the baseline viral load quantified on diagnosis of HCV patients.

Patients and methods

Patients

This study included 147 chronically HCV infected patients recruited from the National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt.

The study has been conducted in accordance with national protocols for the treatment of chronic hepatitis C approved by Institutional Review Board (IRB) for Human Subject Research. This national protocol has been issued by the National Committee for Control of Viral Hepatitis, Ministry of Health in Egypt. This committee is organized and operated according to the Declaration of Helsinki for human subjects (2008). Moreover, an informed consent was obtained from every participant.

The selection criteria for chronic HCV patients were positive for HCV Ab and positive viremia at the time of diagnosis. The exclusion criteria were viral infections, bilharziasis, and any other autoimmune diseases or cancers.

A complete medical history and biochemical parameters were obtained from the patients' files.

Moreover, the METAVIR score which was evaluated in the liver biopsy for all patients before starting the treatment was also obtained from the patient's records. This score helps to determine the degree of liver inflammation and fibrosis. It is a semi quantitative scoring system described by Scheuer [24]. The degree of inflammation was graded on a scale of 0–4 (0: absent; 1: minimal; 2: mild; 3: moderate; 4: severe). The degree of fibrosis was staged on a scale of 0–4 (0: absent; 1: mild without septa; 2: moderate with few septa; 3: numerous septa without cirrhosis; 4: cirrhosis).

Blood sample and DNA isolation

A peripheral venous blood sample of 2 ml was drawn from each individual. The blood sample was collected in sterile anticoagulant tubes. Genomic DNA was extracted from EDTA whole blood using Pure Link® Genomic DNA Mini Kit; Invitrogen, USA.

Genetic polymorphism detection

One SNP (rs10754558) within NLRP3 gene was genotyped in this study. The genotyping was performed using TaqMan Predesigned SNP Genotyping Assay; Invitrogen, Applied Biosystem, USA and TaqMan Universal Master Mix II, no UNG; Invitrogen, USA.

The PCR for detection of SNP was carried out according to the manufacturer's instructions using Rotor-Gene® Qiagen, real time PCR, USA. Allelic discrimination was performed using Rotor –Gene Q Software version: 2.0.2 (Build 4).

Statistical analysis

Statistical analyses were conducted by software package SPSS version 21 (SPSS, Chicago, IL, USA). Continuous variables of demographic and anthropometric characteristics were compared by the use of Student's t test while for more than 2 groups ANOVA test was computed. The genotype and allele frequencies were calculated using gene-counting method. Univariate comparisons of categorical variables were performed with Chi–square test. Allele frequencies and genotype distributions between the studied groups were compared by chi square test and Fisher's exact test when expected value < 5 were found for any cell. The nominal level of statistical significance for all analyses was $p < 0.05$.

Results

Patients' characteristics are listed in table 1. The genotype distributions of the HCV patients were in Hardy–Weinberg equilibrium. The assessment of the impact of genotype and allele frequencies of NLRP3 C-29940G polymorphism on the degree of liver fibrosis and liver inflammation are shown in table 2 and 3 respectively. Moreover, the relation between the degree of liver fibrosis and the baseline viral load is shown in table 4. No significant difference in genotype distribution or allele frequencies was found neither in the degrees of liver fibrosis nor in degrees of liver inflammation. In addition , no significant effect was manifested between the serum viral load and the degree of liver fibrosis

Table 1: Patients' characteristics

Sex	**Male**	**94 (63.9%)**
	Female	53 (36.1%)
Age		41 ± 10
BMI		27 ± 4
ALT		57±28
AST		48±24
αFP		9.5±17.5
Degree of Fibrosis	**F1**	89 (60.5%)
	F2	30 (20.4%)
	F3	28 (19%)
Degree of Inflammation	**A0**	1 (0.7 %)
	A1	105 (71.4 %)
	A2	32 (21.8 %)
	A3	5 (3.4 %)
	A4	4 (2.7 %)

BMI= Body mass index, ALT= alanine transaminase, AST= aspartate transaminase, αFP= alpha-feto protein, quantative data expressed as mean ± S.D.

Table 2: Impact of allele and genotype frequencies of NLRP3 gene C-29940G (rs10754558) polymorphism on the degree of liver fibrosis.

	F1 (N=89)	**F2 (N=30)**	**F3 (N=28)**	**P-value**
CC	17(19.1%)	5 (16.7%)	8(28.6%)	0.47
CG	47(52.8%)	16(53.3%)	15(53.6%)	0.99
GG	25(28.1%)	9(30%)	5(17.9)	0.5
C	81(45.5%)	26(43.3%)	31(55.4%)	0.36
G	97(54.5%)	34(56.7%)	25(44.6%)	0.36

The statistical analyses were conducted using Chi-square test

Table 3: Impact of allele and genotype frequencies of NLRP3 gene C-29940G (rs10754558) polymorphism impact on the degree of liver inflammation.

	A0-A1 (N=106)	A2 (N=32)	A3-A4 (N=9)	P-value
CC	21(19.8%)	7(21.9%)	2(22.2%)	0.96
CG	55(51.9%)	19(59.4%)	4(44.4%)	0.66
GG	30(28.3%)	6(18.6%)	3(33.3%)	0.5
C	97(45.8%)	33(51.6%)	8(44.4%)	0.7
G	115(54.2%)	31(48.4%)	10(55.6%)	0.7

The statistical analyses were conducted using Chi-square test

Table 4: Relation between the degree of liver inflammation and the extent of the initial viral load

Metavir (degree of fibrosis)	Log (Viral Load)		ANOVA result
	Mean	S.E. of Mean	P-value
F1	5.49	0.1	0.467
F2	5.7	0.15	
F3	5.4	0.15	

The statistical analyses were conducted using ANOVA test

Discussion

Hepatic inflammation during chronic HCV infection is the key player in progression of liver disease and consequently development of liver cancer [12]. Inflammasome are molecular platforms known to be involved in recognizing pathogens and eliciting the innate immune response [14]. Notably, IL-1β and the NLRP3 inflammasome has been found to be essential factors for dendritic cell maturation, antigen presentation and T cell activation[25-27]. This is supporting the notion that NLRP3 inflammasome modulates the acquired immunity as well as the innate immunity against several pathogens. Recently, numerous studies have demonstrated that NLRP3 inflammasome is a cytoplasmic sensor of HCV which upon activation recruits several proteins in multiprotein complex resulting in the activation of caspase-1 and maturation of IL-1β. Interestingly this role of NLRP3 in innate immune response against HCV have been reported in different types of cells including monocytes hepatocytes and liver macrophages [15-16,28].. Moreover, it has been discovered that IL-1β production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease [15]. Thus the objective of the current study was to investigate the role of a genetic variation within NLRP3 gene (rs10754558) in the degree of liver fibrosis and inflammation Egyptian patients chronically infected with HCV. The studied single nucleotide polymorphism (rs10754558) was selected based on the previously published literatures showing that the G allele of this 3'UTR SNP enhances mRNA stability [29] that secures more active NLRP3 protein[30-31]. NLRP3 (rs10754558) polymorphism was not associated neither with degree of liver fibrosis nor with the degree of liver inflammation. Because the allele and genotype frequency distribution of NLRP3 (rs10754558) were similar within different groups of liver fibrosis and inflammation. Moreover, the allelic

distribution of NLRP3 (rs10754558) did not vary significantly with the initial viral load quantified at the diagnosis. Notably, the current study has been conducted on the Egyptian patients with HCV genotype 4a. So the findings of our study does not exclude associations between genetic variation in NLRP3 (rs10754558) and the degree of fibrosis and inflammation in other populations with different HCV genotypes.

Conclusion

The selected 3'UTR SNP (rs10754558) in NLRP3 gene is affecting the mRNA stability and consequently the intracellular NLRP3 protein level. Yet, we could not find any significant association for this SNP with the degree of liver fibrosis and inflammation in Egyptian HCV patients. Nevertheless, the crucial role played by the NLRP3 inflammasome in the immune response against HCV infection necessitates further investigations for other polymorphisms within NLRP3 gene to unveil their role of in HCV infection and disease outcome in the chronic HCV patients.

Competing interests

The authors declare that they have no competing interests to disclose.

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