

Molecular Biological Studies on Immune Responses Enhancement against HCV, the Role of Toll like Receptor

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ABSTRACT

Epidemic disease with hepatitis C virus (HCV) was raised by scanty immune restraint. Empirical facts propose that low activation of TLRs 2-4 sensors participates to epidemic disease. We predestined the allocation of Single nucleotide Polymorphism (SNPs) in the TLR2 Arg677Trp in epidemic HCV disease and minus HCV sets. This research purposes to discuss the modulation of genetic ethnic polymorphisms of TLR2 gene in the epidemic HCV disease Egyptian positive HCV. TLR2 Arg677Trp rs121917864 (C/T) were genotyped in 549 positive HCV and 519 negative HCV sets. The combination analysis proved that the having of rare allele T of TLR2 Arg677Trp could have a low significant risk for epidemic HCV disease (OR= 1.5206 95%CI 1.1521 to 2.0069) and P = 0.0031.

Keywords: Polymorphism, TLR, HCV and SNPs.

INTRODUCTION

Epidemic hepatitis C virus (HCV) disease is a big reason behind epidemic liver disease result in advanced fibrosis of liver that leads to cirrhosis of liver after long term progress and hepatocellular carcinoma (HCC) (Bertino *et al.* 2016). About 20% of men and women Infected with hepatitis C virus able to eradicate this problem automatically, while the majority become chronic contaminated (Naggie 2012). The natural immune system plays a vital role in disease emergence of hepatitis C Virus the invasion hosted by infection with hepatitis (C), the natural immunity through the identification of the virus from PRRs (Pattern Recognition Receptor) (Martínez *et al.* 2016). The hepatitis C virus different effects after activating the route would enhance the TLR pathway which could lead to the production of cytokine result in liver damage device and avoid reactions immune system to prepare the viral load. (Howell *et al.* 2013). Toll-like receptor (TLR) is the best studied the category of PRRs recognize germs specific molecular patterns of various organisms. (Jenne and Kubes 2013) Lipopeptide operations with hepatitis C Virus been found essential protein to induce the innate resistant response via TLR2 and TLR4, Several studies have shown that genetic TLR may affect susceptibility to infection (Martínez *et al.* 2016).

It has been well documented that the TLRs inside cells is included in the identification of the virus, such as 24 nucleotides and the opening of opening of the reaction of the interferon anti-virus (Akira *et al.* 2006). Express cellular TLRs surface such as TLR2 TLR4, play an important role in the defense against host viruses such as herpes simplex virus host, cytomegalovirus virus, respiratory syncytial virus and measles virus (Boehme and Compton 2004). The hepatitis C virus indicate the proteins to regulate TLR2 and TLR4 appearance on the peripheral blood mononuclear cellular articles and Raji cells , then modify the cellular proinflammatory response (Machida *et al.* 2006). The studies conducted recently that the core of HCV can induce TLR2 (Dolganuc *et al.* 2006).

MATERIALS AND METHODS

Study Subjects. In this study 549 HCV contaminated patients and 519 healthy control all patients and control

cases were employed from Clinics of Tropical Medicine Department, Mansoura University during the period from January 2013 to Mar 2015.

Biochemical analysis: Almost all of the epidemic HCV and the negative HCV cases were diagnosed with HCV, based on antibodies of anti HCV, HCV RNA by PCR. Also, lab investigations were performed for all those serum cases.

Tests for synthetic function: Bilirubin (Total & Direct), Serum albumin Prothrombin time and concentration enzymes alanine amino transferase, aspartate amino transferase and alkaline phosphates (Table 1).

DNA Extraction: Genome of deoxyribonucleic acid was abstracted from leucocytes of peripheral blood samples by using a commercial Qiagen DNA isolation kit (Qiagen, Germany), was agreed to the instructions of industrialist, This extracted DNA was used for real time PCR.

Table 1. Baseline clinical and laboratory characteristics of the studied groups.

	HCV (549)	Control (519)
	Mean (±SD)	Mean (±SD)
Age	43 (12.7)	36 (22.7)
S. Bilirubin mg/dl	1.5 (0.7)	0.8 (0.2)
S. Albumin gm/dl	3.9 (0.5)	4.4 (0.5)
SGOT IU/L	49 (10)	17(5)
SGPT IU/L	52 (11)	19(8)
ALP U/L	77.8 (15.9)	55 (18.9)
AFP ng/ml	25 (16.8)	7(3.1)
ANA (U)	0.7 (0.18)	0.6 (0.25)
S. Creatinine (mg/dl)	0.8 (0.35)	0.6 (0.39)
S. Cholesterol(mg/dl)	157 (25.9)	149 (24.2)

Allelic discrimination (AD) with real time PCR

This SNP was prepared as primer for TaqMan allelic discrimination. A fluorescent dye (VIC and FAM) of SNP probe was used in the SNP polymorphism of each DNA sample. Real-time PCR reaction was made on each sample the system (model 7500, Biosystems Applied) was used a fluoresce readymade categorized SNP primers and probes (was bought, from Biosystems Applied). A similar fluorescent dye detector to the wild type (allele 1) and the other similar fluorescent dye detector is to the modified (allele 2).

Genotyping of certain probes (SNPs) were CC, CT, and TT. to genotype variants at the SNP site in the

wanted sequence were used 2 primer and probe pairs in each reaction.

The FAM dye was labeled probe for allele C, the VIC dye was labeled probe for allele T.

The connection between sequences and signals fluorescence in the sample.

Homozygosity of allele 1 is CC leading to substantial increase in fluorescence only VIC[®] dye.

Homozygosity of allele 2 is TT leading to substantial increase in fluorescence only FAM[™] dye.

Heterozygosity of allele 1 and allele 2 are CT leading to substantial increase in both fluorescence signals (VIC[®] and FAM[™] dye).

PCR Reagents used in the reaction: Universal Master Mix II (2x) from TaqMan[®] Supplied from (Biosystems Applied) and stored at 0-20 C up to be used.

Genotyping Assay Mix (20x) the probe for TLR2 Arg677Trp rs121917864 (C/T) amplification from TaqMan[®].

DNA Template + RNase free water.

Components of the mix of PCR reaction for the SNPs readymade.

10.0 µl of Master Mix Universal II 2X,

1.0 µl of Genotyping SNP Assay Mix 20x,

1.0 µl of Template of DNA its concentration from 1 to 100 ng and

8.0 µl of Free water the total volume of the sample was 20- µl.

preparation Procedures of run:

95 °C for 10 minutes hold. 40 cycles of:

first step Denaturation at: 95 °C for 15seconds.

Second step Annealing at: 60 °C for 1 minute

Third step Extension at: 60 °C for 1 minute.

Record Analysis: Genotypic Allelic frequencies, estimated direct screening Pearson chi-square (X²) tests to determine the importance of the relationship between the relevant groups 2x2 emergencies. He expressed the results in conditions quite different from the odds ratio (or) 95% confidence intervals (95%) and P values. Odds Ratios calculated way for the method of wolf. The decisive moment is zero, Haldane to modify the application of the equation wolf using the formula: $Or + 1/2 = [(x \text{ after} + 1/2)(d)]$ as well as $+ 1/2](b)(10)c + 1/2]$, a, b, c, and d represents the afflicted individuals and the risk of non-affected individuals, on his part, having the risk of suffering individuals who, on his part, for his part, however, the risk of non-affected individuals, on his part, risk, correspondingly. $AP \leq 0.05$ used to be statistically significant. All standard analysis using SPSS (version 17.0.0, SPSS). The Test SNPs were in Hardy-Weinberg equilibrium HWE, and a sharp of p value of 0.01 was set.

RESULTS

The allele carriage of rs121917864 (C/T) polymorphism is 390 and 418 of CC allele in HCV patients and negative control respectively, 138 and 92 of CT allele carriage in HCV patients and negative control respectively and 21 and 9 of TT allele carriage in HCV patients and negative control respectively. The allele frequency of C allele was 83.6% in HCV patients

and 89.4% in negative control and the allele frequency of T allele was 16.4% in HCV patients and 10.6% in negative control. The allele frequency, PIC and Heterozygosity of this SNP TLR2 (rs121917864) (C/T) are polymorphic enough for doing genetic analysis. These SNP are in Hardy Weinberg indicating that the selected prop are represent their population genetically (Table 2).

Table 2. The TLR2 rs121917864 polymorphism C/T Distributions

	Positive HCV (549)	Negative HCV (519)
Wild type CC	390	418
Heterozygous CT	138	92
Rare allele TT	21	9
Allele frequencies		
C	83.6%	89.4%
T	16.4%	10.6%
PIC	0.272	0.194
Homozygote	74.8%	82.2%
Heterozygote	25.2%	17.8%
Hardy-Weinberg χ^2		
X ²	3.782657	2.159065
P	0.051786	0.141731

This table show the allele carriage of T allele of TLR2 rs121917864 is significantly lower in HCV group compared to Control group with (OR=1.5206 95% CI 1.1521 to 2.0069) and P = 0.0031 and risk for persistent HCV infectin. This indicates the genotyping CC carriage is a protective variable against HCV infection (Table 3).

Table 3. Association of the allele T of TLR2 rs121917864 CT polymorphism and HCV infection

	Positive vs. Negative
Odds ratio (OR)	1.5206
95% CI:	1.1521 to 2.0069
z statistic	2.960
Significant level	0.0031
CP	0.0093

DISCUSSION

Hepatitis C causes major health problems worldwide, as virus infection can lead to serious liver damage, including liver cirrhosis and liver cancer (Seeff 2002). Many studies have shown that the differences in genetics host can modify of hepatitis. For example, showing a polymorphism in the IL28B genes to influence the efficiency of treatment depend on interferon (Clark and Thompson 2012) and clear the virus.

TLRs were termed in humans. TLR2, TLR4, and TLR6 were proven to connect to microbes, lipoproteins, glycoproteins, and peptidoglycans. Through the first infection steps, share these receptors in the confession of such antigens microbes. DNA or RNA genomic of several pathogens, including viruses and bacteria protozoa, remote sensing of the recognition of TLR9, it

is also able to modify the DNA types such as islands, its position (CPG) toward unmethylated. Stranded RNA and double stranded RNA have been identified from infection such as West Nile virus, respiratory syncytial virus, HIV and influenza virus, by TLR8, TLR7 and TLR3. Although the exact function of the TLR10 is not fully explained, it is thought that the compounds resemble profilin (O'Neill *et al.* 2009). The occurrence of core and NS3 proteins of HCV is as signals of TLR2 has shown in previous studies (Eid *et al.* 2007). The in vitro model viral hepatitis induced inflammation protein in cells to activate the account from TLR2 receptor cells are incomplete (Eid *et al.* 2007).

Recently study was reported that TLR2 has been induced by tumor necrosis factor alpha. HCV infection or viral E2-CD81 interaction is a reason of tumor necrosis factor alpha production. In vivo TLR2 was induced by oblique results of other viral protein. Even though in the obscurity of the modification in the level of expression, it is possible that other TLRs functions can be modified in the hepatitis C virus infection by changing the transfer pathway signal transduction from these TLRs (Machida *et al.* 2006). A heterodimer with TLR1 or TLR6, of TLR2 identifies a large range of common bacterial motif, including lipopeptide, peptidoglycan, glycosylphosphatidylinositol linked protein and zymosan. SNP in TLR2 have recently been associated to human diseases (Akira *et al.* 2001). A C>T Mutation in nucleotide 2029(rs121917864), which replaces Arg677 with Trp is prevalent in African and Asian multitude, but appears to be absent among other multitude. This SNP has just lately been proven to inhibit both Mycobacterium leprae- and M. tuberculosis-mediated NF- κ B activation and production in vitro (Kang *et al.* 2002). In a Korean and a Tunisian multitude, this SNP was associated with leprosy and susceptibility to tuberculosis respectively. This is consistent with the data that patients with this allele show lower main and microbactrium-stimulating serum IL-12 levels, which is required to activate the pathway of IFN- γ and induce T helper response (TH1) against pathogenic intra microphages (Skevaki *et al.* 2015). This information was proved that TLR2 gene polymorphisms have been caused increased susceptibility to infections by TLR2 agonist's bacteria. The allele frequency of the C/T genotype in positive HCV is significantly higher than that in negative control subjects (25.2 versus 17.8%; P 0.0031). Until now, the link between these disease state heterozygous statuses was reported by the hepatitis C virus, and then detection of this polymorphism among positive HCV may provide important information for the analysis of risk profiles regarding susceptibility to HCV. As unknown risk factors may interfere with this presentation, a study of healthy household contacts in the negative HCV is justified. As a result of our work were mixed, with Korea report found alleles TLR2 Arg677TRP recovering were not detected in any patient, indicating that it is rare and incredibly Korean multitude. The results do not allow any conclusions with regard to their work in the development of liver cirrhosis (Kim *et al.* 2010). But another study shows

that the wild-type TLR2 genotype may be a risk factor for cytomegalovirus infection duplication in adult patients (Jabłońska *et al.* 2014).

CONCLUSION

The carriage of rare allele A of TLR2 Arg753Gln would have a low significant risk for consistent of HCV infection.

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**دراسات بيولوجية لاستثارة الجهاز المناعي ضد فيروس سي (دور مستقبلات شبيهة التول)
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فيروس الالتهاب الكبدي سي هو فيروس (RNA) صغير مغلف احادي النطاق ايجابي الاتجاه وهو يؤثر علي الملايين من الناس في جميع انحاء العالم وهو سبب رئيسي لامراض الكبد بما في ذلك تليف الكبد وسرطان الكبد وذكرت دراسة حديثه ان جين مستقبل شبيه التول 2 فيه تعدد جيد للاشكال الوراثية وتم التنبؤ بارتباطه بالحمايه من تليف الكبد بين القوقازيين وتهدف هذه الدراسه الي بحث ارتباط تعدد الاشكال الجينيه لجين مستقبل شبيه التول 2 بالمرضي المصابين بالفيروس وقد تم توضيح الطراز الجيني لهذه الجينات لعدد 549 فرد مصاب بالفيروس و519 فرد غير مصاب اكد تحليل الارتباط ان جميع الجينات اظهرت اختلافا كبيرا في توزيعها بين المرضي المصابين بالفيروس وغير مصابين (المجموعه الضابطه) حيث كان هذا الاليل وقائي ضد الاصابه بالتهاب الكبد الفيروسي سي و الاليل المحور ترتبط فيها بازدياد خطورة الاصابه بالتهاب الكبد الفيروسي سي في الاشخاص الحاملين لهذه الاليلات في العائلات المصريه **الكلمات الداله:** التهاب الكبد الفيروسي و جين مستقبل شبيه التول والتحورات الجينيه