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(ORIGINAL ARTICLE)

Impact of Interleukin-28 b (rs12979860) Gene Polymorphism on Intrafamilial Transmission of HCV in Egypt

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ABSTRACT

Background: Intra-familial transmission of hepatitis C virus (HCV) in Egypt was found to play a major role in the high prevalence of HCV in Egypt. A single nucleotide polymorphism (SNP) near the interleukin-28B *(IL28B) (rs12979860)* gene is associated with self-cure of Hepatitis C Virus (HCV). The aim determining the significance of *IL-28B (rs12979860)* gene polymorphism in intra-familial transmission of HCV in Egypt**.**

Methods: Three hundred HCV patients, 860 family members and 100 healthy subjects were studied. HCV antibodies test was done by Enzyme Linked Immunosorbent Assay (ELISA) for all family members. Real-Time Polymerase chain reaction (PCR) ascertained presence of chronic HCV. PCR and restriction enzyme analysis were used to determine the molecular structure of *IL-28B (rs12979860)* gene to all studied subjects.

Results: The distribution of *IL-28B (rs12979860)* gene polymorphism in patients was 24.3%, 53.7% and 22% for C/C, C/T and T/T genotypes respectively, in infected family members was 27.5%, 54.9% and 17.6% for C/C, C/T and T/T respectively and in negative family members was 28.5%, 54.1% and 17.4% for C/C, C/T and T/T respectively. C/C genotype was significantly more frequent in non-infected family members while T/T genotype was significantly more frequent in HCV infected patients when compared with controls.

Conclusions: Our study concluded that *IL-28B (rs12979860)* gene polymorphism has no impact on intra-familial transmission of HCV.

Key words: Egypt; HCV; *IL-28B*; intra-familial

INTRODUCTION

Seventy million people all over the world suffer from chronic hepatitis C Virus (HCV) infection and the disease may be complicated by liver cirrhosis and hepatocellular carcinoma [1]. Genotype 4 represents 92.5% of HCV patients in Egypt [2]. Ten percent of Egyptians test positive for HCV antibodies and 7% are HCV Polymerase Chain Reaction (PCR) positive [3]. The high prevalence of HCV in Egypt may be related to intra-familial transmission of HCV [4]. Plancoulaine et al, 2008 [5] revealed that HCV infection in countries with high prevalence has a familial component that may be explained by genetic susceptibility to infection. A single nucleotide polymorphism (SNP) near the *interleukin-28B (IL28B) (rs12979860)* gene is associated with self-cure of HCV [6]. The host innate immune response is partially responsible for the relation between polymorphism of *IL28B* gene and viral clearance. *IL28B* encodes Interferon Lambda 3 (IFN-λ3), which is involved in viral control, including HCV. *IL28A&B and IL29* are three closely related cytokine genes that encode proteins known as type III Interferons (IFNs) at chromosomal region 19q13. The three cytokines are induced by viral infection and have antiviral activity [7]. In this study we evaluate the role of *IL-28B (rs12979860)* gene polymorphism in facilitating or protecting against intra-familial transmission of HCV in Egypt.

METHODS

Patients and groups

Three hundred Egyptian patients with chronic HCV, 860 subjects of their family members and a control group (no = 100) were included in this study. All patients were recruited from Tropical

Medicine Department, Tanta University Hospital, Tanta, Egypt. Inclusion criteria were; positive HCV antibodies by ELISA and HCV RNA by reverse transcriptase polymerase chain reaction (RT- PCR) of all HCV infected subjects, age ≥18 years old, age of family member ≥ 3 years old, one year period or more of HCV infection in the HCV infected patients. Hundred healthy subjects with normal liver biochemical tests and negative HCV antibodies were included as control group. Subjects of all groups had negative hepatitis B virus (HBV), they were free from other chronic liver and systemic diseases as diabetes mellitus. Drug history of subjects of all groups revealed that they were not on corticosteroids, cytotoxic or immunosuppressive medications.

The studied groups were as following:

Group I: 300 HCV infected patients.

Group II: 91 HCV positive family members.

Group III: 769 HCV negative family members.

Control group: 100 healthy subjects.

Ethics approval and consent to participate

All participants gave their informed written consents and the study was approved by the local ethics committee of Tanta Faculty of Medicine, Tanta, Egypt, the committee's reference number is not available. Objectives, protocols and procedures of this study were according to the Helsinki declaration.

Clinical examination and investigations

Patients and their infected family members underwent history taking, clinical examination, liver biochemical tests, complete blood picture, fasting blood sugar, antinuclear antibody, hepatitis B surface antigen and pelvi-abdominal ultrasonography.

Diagnosis of HCV

HCV antibodies were detected by third-generation assay (Abbott IMx, Abbott Diagnostics, Maidenhead, UK) [8]. Patients were tested for HCV RNA by a commercially available assay (Amplicor, Roche, Basel, Switzerland).**[9]**

Molecular study: *IL-28B (rs12979860)* gene polymorphisms

The extraction of Genomic DNA from peripheral blood leukocytes was done by using Gene JET ™Genomic DNA Purification Kit (Fermentas, German) following the manufacturer instructions. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used in Genotyping of the rs12979860 C/T polymorphisms in the *IL28B* gene as described by Fabris et al., 2011 [10].

STATISTICAL ANALYSIS

The mean ± SD, frequencies (number) and percentages were used when appropriate Normal data of the numerical variables between groups were compared using one-way analysis of variance test while the Kruskal–Wallis test was used when the data were not normal and qualitative data compared with chi square test. P-values less than 0.05 were considered statistically significant. computer program SPSS (Statistical Package for the Social Science; SPSS, Chicago, IL, USA) version 15 for Microsoft Windows was used to perform statistical calculations.

RESULTS

Ninety-one (10.58%) of 860 family members had positive HCV PCR while 769 (89.42%) were HCV negative. As regards sex and age of the 300 HCV patients; the males were 146 (48.67%) and the mean age was 42.69±12.5 years. Forty one (45%) of HCV positive family members were males and the mean age was 39.67±14.4 years while in HCV negative family members, males were 337 (44%) and the mean age was 27.32±15.5 years. Males were 47 (47%) of controls and the mean age was 38.71±9.3. We found no statistical difference between groups as regards gender (P> 0.05).The age of subjects in HCV negative family members was less when compared to the other groups (P < 0.05).

Comparisons of genotype frequencies among the three groups showed a significant difference P < 0.05 while comparisons of allele frequencies was statistically insignificant P > 0.05(Table1). C/C genotype was significantly more frequent in negative family members when compared to control group P < 0.05 (Table 2).The frequency of C/T genotype was significantly higher in controls when compared to HCV infected patients, negative and positive HCV family members P < 0.05 (Table 3). T/T genotype was significantly more frequent in HCV infected patients when compared to control group P < 0.05 (Table 4).

Table(1): The frequency of IL-28B (rs12979860) genotypes and alleles among the studied groups

Groups	Genotype						Allele			
	C/C		C/T		T/T		C		T	
	No.	%	No.	%	No.	%	No.	%	No.	%
HCV infected patients (n=300)	73	24.3	161	53.7	66	22	307	51.2	293	48.8
HCV positive family	25	27.5	50	54.9	16	17.6	100	54.9	82	45.1

Groups	Genotype						Allele			
	C/C		C/T		T/T		C		T	
	No.	%	No.	%	No.	%	No.	%	No.	%
members (n=91)										
HCV negative family members (n=769)	219	28.5	416	54.1	134	17.4	854	55.5	684	44.5
Controls(n=100)	16	16	74	74	10	10	106	53	94	47
p-value	0.004*						0.3			

P < 0.05 *significant.

Table (2): The distribution of IL-28B (rs12979860) C/C genotype in the studied groups

Groups	C/C genotype of IL-28B gene					
	Present		Absent		Total	
	No.	%	No.	%	No.	%
HCV infected patients	73	24.3	227	75.7	300	100
HCV positive family members	25	27.5	66	72.5	91	100
HCV negative family members	219	28.5 *	550	71.5	769	100
Controls	16	16	84	84	100	100
Total	333	26.4	927	73.6	1260	100

P < 0.05 *significant. Genotype C/C in the groups(X^2= 7.9, P= 0.04*). Genotype C/C was significantly more frequent in negative family members compared with controls (X^2=6.9, P=0.008*).

Table (3): The distribution of IL-28B (rs12979860) C/T genotype in the studied groups

Groups	C/T genotype of IL-28B gene					
	Present		Absent		Total	
	No.	%	No.	%	No.	%
HCV infected patients	161	53.7	139	46.3	300	100
HCV positive family members	50	54.9	41	45.1	91	100
HCV negative family members	416	54.1	353	45.9	769	100
Controls	74	74*	26	26	100	100
Total	701	55.6	559	44.4	1260	100

P < 0.05 *significant. Genotype C/T in the groups(X^2= 14.8, P= 0.001*).Genotype C/T was significantly more frequent in controls compared with other groups (p<0.005*).

Table(4): The distribution of IL-28B (rs12979860) T/T genotype in the studied groups

Groups	T/T genotype of IL-28B gene					
	Present		Absent		Total	
	No.	%	No.	%	No.	%
HCV infected patients	66	22*	234	78	300	100
HCV positive family members	16	17.6	75	82.4	91	100
HCV negative family members	134	17.4	635	82.6	769	100
Controls	10	10	90	90	100	100
Total	226	17.9	1034	82.1	1260	100

P < 0.05 *significant. Genotype T/T in the groups(X^2= 7.7, P= 0.051).Genotype T/T was significantly more frequent in HCV infected patients compared with controls (X^2=7.1, P=0.008*)

DISCUSSION

Host genetics affect cellular immune reaction especially T helper (Th) cells of Th1 type that play role in HCV clearance [11]. *IL-28B (rs12979860)* is a Th1 type cytokine, a member of type III Interferons (IFNs). *IL-28B (rs12979860)* shares in the regulation of intracellular IFN evoked gene expression [12]. *IL-28B (rs12979860)* has antiviral activity and facilitates natural clearance of HCV [13]. In the present study *IL-28B (rs12979860)* genotype distribution in HCV infected patients was (C/C = 24.3%, C/T = 53.7% and T/T = 22%) and allele frequency was 48.8 % for T allele and 51.2% for C allele. These results agree with Kurbanov et al, [14] who concluded that *IL-28B (rs12979860)* genotype distribution in Egyptian patients with chronic HCV was 30.5 %, 54.9 %, and 14.6 % for C/C, C/T, and T/T genotypes, respectively and allele frequency was 57.9 % for C allele and 42.1% for T allele and with Hendy et al, [15] who found that *IL-28B (rs12979860)* genotype distribution in Egyptian chronic HCV patients was 39%, 51%, and 10% for C/C, C/T, and T/T genotypes, respectively. Similarly, a recent study of an Uruguayan HCV-infected cohort revealed that the percentage of the rs12979860 genotypes were 29.5% CC, 47.4% CT and 23.1% TT [16]. El-Awady et al, [6] reported that genotype and allele percentage of *IL28B (rs12979860)* in Egyptian chronic HCV patients was 13%, 75%, and 12% for C/C, C/T, and T/T genotypes, respectively and allele frequency was 50% for C allele and 50% for T allele. This difference in genotype distribution percentages may be due to presence of liver cirrhosis in studied subjects of the latter study while our study included patients with chronic hepatitis C without cirrhosis.

In our study, T/T genotype was significantly more frequent in HCV infected patients when compared to controls (22% vs. 10% respectively). No significant difference was detected between patients, positive family members in one hand and negative family members in the other hand, and so this cannot confirm that the presence of T/T genotype of *IL-28B (rs12979860)* gene increases the susceptibility for HCV infection. These results agree with El-Awady et al, [6] who reported higher frequencies of *IL-28B (rs12979860)* T/T genotype in patients with chronic HCV and liver cirrhosis than in healthy controls. Fabris et al, [10] reported that in chronic HCV patients, the T/T genotype was more frequent in patients with liver cirrhosis than in those with chronic hepatitis. TT genotype may favor the progress of liver disease from chronic hepatitis to cirrhosis.

In our study, a significant increase in the incidence of C/C genotype of IL-28B gene in negative HCV family contacts compared with controls was observed (28.5% Vs 16%). However, there was non- significant difference between the positive and negative HCV family members, and so we could not prove in our study that the presence of C/C genotype of IL-28B gene have a protective effect against HCV infection. El-Awady et al, [6] who observed that frequencies of *IL-28B (rs12979860)* C/C genotype was higher in spontaneously cured patients than in healthy controls, patients with liver cirrhosis due to HCV infection. Thomas et al, [17] found that *IL28B* (rs12979860) was significantly related to clearance of HCV spontaneously in African or European ancestry. Molecular study of *IL-28B (rs12979860)* genotypes in a recent study in Egypt revealed that the CC genotype in resolved patients was higher than patients with chronic HCV ($p = 0.011$, OR = 0.48, 95% CI = 0.269–0.850) [18].Another recent study in Iran concluded that the CC genotype percentage of IL-28B (rs12979860) was higher in patients who cured from HCV without treatment than in those with chronic HCV (65.1% vs 34.92%) [19]. All the above studies differ from our study because they investigated C/C genotype in spontaneously cured patients while our study investigated the role of C/C genotype in the protection against intra-familial HCV infection.

In the present study, the frequency of C/T genotype was significantly higher in controls when compared to HCV infected patients, negative and positive HCV family members (74% vs 53.7%, 54.1% and 54.9% respectively). In contrast, Echeverría N et al, concluded that IL 28 B (rs12979860) genotypes frequencies within the control group were 45.7% CC, 42.4% CT and 11.9% TT, these different results may be due different ancestry and presence of HCV genotype 1in the studied subjects of the latter study [16].

CONCLUSION

Our study demonstrated that C/C genotype for *IL-28B (rs12979860)* gene has been significantly expressed in non-infected family members of HCV patients when compared to controls but no significant difference was detected when compared to patients and positive family members. T/T genotype for *IL-28B (rs12979860)* gene has been significantly expressed in HCV infected patients when compared to controls but no significant difference was detected when compared to negative family members. These above results could not prove that C/C genotype is protective against intra-familial transmission of HCV or that T/T genotype can facilitate it. Our study concluded that *IL-28B (rs12979860)* gene polymorphism has no impact on intra-familial transmission of HCV.

ABBREVIATIONS

HCV, Hepatitis C virus; SNP, Single nucleotide polymorphism; IL-28B, Interleukin 28 B; IFN-λ3,

Interferon lambda 3;IL 29, Interleukin 29; IFNs, Interferons; ELISA, Enzyme linked immunonosorbent assay; RNA, Ribonucleic acid; RT- PCR, Reverse transcriptase polymerase chain reaction; HBV, Hepatitis B virus; DNA, Deoxyribonucleic acid; PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; dNTP, deoxyribose nucleotide triphosphate.

Declaration of interest

The authors report no conflicts of interest.

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Authors' Contributions

NE: initiated the project, designed and implemented the study for application analyzed the data, drafted and revised the paper. FA: analyzed the data, drafted and revised the paper. LA: was responsible for acquisition, analysis and interpretation of data. SS was responsible for acquisition, analysis and interpretation of data. LE: performed laboratory and molecular investigations. KS: performed laboratory and molecular investigations. MA: performed laboratory and molecular investigations. RE analyzed the data, drafted and revised the paper. All authors have read and approved the final article.

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