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Lack of association between interleukin 28B *rs12979860* gene polymorphism and hepatocellular carcinoma development in chronic hepatitis C

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Discovery of hepatocellular carcinoma (HCC)-related single nucleotide polymorphisms (SNPs) may enable patients at risk to adapt their lifestyle and legitimate implementation by their doctors of surveillance programs facilitating early diagnosis and subsequent disease management. This study aimed to investigate whether interleukin-28B (IL-28B) *rs12979860* SNP affect the development of HCC in chronic hepatitis C (CHC) infection. IL-28B *rs12979860* was genotyped in 80 CHC patients (30 with liver cirrhosis and 50 with HCC). T allele carriers (CT and TT combined) were slightly more frequent in HCC patients (39/50, 78%), without any significant difference (*P*=0.437), compared with patients with liver cirrhosis (21/30, 70%). ROC curve analysis showed that there is no good reliable predictive power for IL-28B T allele to predict the HCC development (AUC=0.540). When comparing patients with CC genotype with T allele carriers, biochemical data did not show any significant differences (*P*>0.05). Also, Spearman correlation analysis revealed that there was no association between IL-28B SNP and any of biochemical parameters. Moreover, there was no significant difference (*P*>0.05) in IL-28B *rs12979860* genotypes distribution between patients with low and those with high AFP levels and between patients with single and those with multiple tumors. In conclusion, we did not find any significant association between IL-28B *rs12979860* SNP and the development of HCC in Egyptian patients with chronic hepatitis C infection.

Keywords: Chronic hepatitis C, Hepatocellular carcinoma, Development, Interleukin-28B, Polymorphism

INTRODUCTION

Globally, each year more than half a million patients with hepatocellular carcinoma (HCC) are diagnosed. It is the 3rd cancer death leading cause worldwide. These figures show that HCC is a grim disease with massive global impact (Attallah et al., 2016). In Egypt, HCC is the 6th most frequent cancer in women and the 2nd most frequent cancer in men. In Egypt, former studies have shown an overall increase in the relative frequency of all liver-related tumors and have shown the increasing importance of hepatitis C (HCV) infection in hepatic tumor etiology (Ziada et al., 2016).

Generally, it is supposed that many factors, including host, viral and environmental elements, are contributed with the development of HCV-associated HCC (Joshita et al., 2012). IL28B gene single nucleotide polymorphisms (SNPs) have been reported as strong predictors of treatment-induced (Balagopal et al., 2010) and HCV spontaneous clearance (Thomas et al., 2009). Although such findings have suggested strong association between antiviral therapy outcome and IL28B SNPs, the association of this genetic variant with the progression of chronic HCV

infection (CHC) is still controversial (Fabris et al., 2011; Kitson et al., 2014). Moreover, it remains unclear whether IL28B SNPs affects the development of HCV-associated HCC (Joshita et al., 2012).

This study aimed to investigate the association IL-28B *rs12979860* with the development of HCV-related HCC. We assessed the allelic and genotypic frequencies of IL-28B *rs12979860* in Egyptian individuals with CHC, compared the allelic distribution between cirrhosis and HCC and investigated the relation between IL-28B *rs12979860* SNP and some tumor features (number of lesions and AFP levels).

MATERIALS AND METHODS

Study population

A total of 80 consecutively collected Egyptian patients with CHC (30 with liver cirrhosis and 50 with HCC) who were seen at Egyptian Liver Research Institute and Hospital (ELRIAH), Dakahlia, Egypt were enrolled. The CHC diagnosis was based on the following criteria: (1) positive for anti-HCV antibody and HCV-RNA for at least 6 months, (2) lack of detectable antibody to the human immunodeficiency virus and hepatitis B surface antigen and (3) the exclusion of other chronic liver disease causes. Diagnosis of cirrhosis was by characteristic clinical signs of advanced hepatic disease and/or histologic examination. HCC was diagnosed by imaging screening and/or histologic examination. Study protocol was conducted in agreement with the ethical principles and guidance of the Helsinki Declaration. All patients provided written consent to participate in the study. Clinical and demographical data were included in a database.

Laboratory tests

All patients were tested for underlying HCV infection (positive for anti-HCV antibodies (Biomedica, Sorin, Italy) and for HCV-RNA (COBAS Ampliprep/ COBAS TaqMan, Roche Diagnostics, Pleasanton, USA). Liver function tests and creatinine were measured using automatic biochemistry analyzer (architect Ci 4100; Abbott diagnostics, Wiesbaden, Germany). Alpha fetoprotein (AFP) level was estimated by chemiluminescence, with Immulite (1000) AFP kit (Diagnostic Products Corporation; Los Angeles, CA, USA). Complete blood count was performed using KX-21 Sysmex automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Transient elastography was carried out by using FibroScan™ (Echosens, Paris, France) according to previously described technique (Sandrin et al., 2003).

Genotyping of IL-28B (*rs12979860*)

According to the manufacturer's instructions, genomic DNA was extracted from whole blood (200 µl) using a QIAamp DNA Blood Mini Kit (QIAGEN, USA). After that, as previously described (Knapp et al., 2011) IL-28B *rs12979860* genotyping was performed using SYBR Green real-time PCR and specific primers.

Statistical Analysis

Percent of C and T alleles of IL-28B *rs12979860* SNP were determined individually by direct counting of the positive individuals for each allele. Differences between groups of patients were evaluated using ANOVA or Student t-test for continuous variables and X^2 test or Fisher exact test for categorical variables. A two-sided *P* value <0.05 was considered as statistically significant. The correlation was evaluated by Spearman's rank correlation coefficient. Receiver operating-characteristic (ROC) curves were constructed for determining the HCC predictive ability of IL-28B SNP in cirrhosis patients. All statistical analyses were performed by SPSS software (SPSS Inc., Chicago, IL) and Graph Pad Prism package (Graph Pad Software, San Diego, CA).

RESULTS

Patient's characteristics

Baseline characteristics of all patients are summarized in Table 1. Univariate analysis was used to assess differences between data of HCC and cirrhosis patients. However HCC patients were older than controls, cirrhotic patients were selected to be age-matched with HCC (*P*=0.09). Patients with HCC produced a range of AFP values from normal to more than 68000 U/L. Moreover data about aspartate and alanine aminotransferases, bilirubin, albumin, alkaline phosphatase, some hematology parameters, platelet count, HCV viral load, creatinine, Fibroscan and tumor nodule status (single or multiple) were included.

Table 1. Comparison between HCC cohort and cirrhosis controls in terms of demographics and clinical characteristics

Parameter [a]	Cirrhosis	HCC	*P* value
Number	30	50	–
Male/female	25/5	36/14	0.290
Age (years)	55.3 ± 1.1	58.1 ± 1.0	0.090
AST (U/L)	69.3 ± 5.9	52.3 ± 4.0	0.020
ALT (U/L)	87.6 ± 10.0	45.3 ± 3.5	<0.0001
ALP (U /L)	85.5 ± 4.1	97.3 ± 3.0	0.020
Total bilirubin (mg/dL)	0.8 ± 0.1	1.6 ± 0.2	0.001
Direct bilirubin (mg/dL)	0.38 ± 0.03	0.83 ± 0.2	0.030
Albumin (g/dL)	4.0 ± 0.1	3.5 ± 0.1	<0.0001
Hemoglobin (g/dL)	14.6 ± 0.27	12.4 ± 0.24	<0.0001
WBCs ($\times10^3$/microliter)	6.2 ± 0.4	4.3 ± 0.2	0.002
RBCs ($\times10^6$/microliter)	4.9 ± 0.8	4.1 ± 0.8	<0.0001
Platelet count ($\times10^3$/microliter)	151.7 ± 8.3	102.5 ± 8.0	<0.0001
AFP (U/L)	–	2539.8 ± 1368.1	–
HCV-RNA ($\times10^5$ IU/ml)	11.1 ± 2.2	4.6 ± 0.8	0.002
Creatinine (mg/dL)	0.8 ± 0.02	0.9 ± 0.03	0.020
Fibroscan (kPa)	24.2 ± 1.6	25.7 ± 1.5	0.519
Focal lesions (Single/multiple)	–	26/24	–

Variables were expressed as mean±SEM; [a] **Abbreviations**: AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; WBCs: white blood cells; RBCs: red blood cells; AFP: alpha-fetoprotein. *P* value for cirrhosis versus HCC (*P* <0.05 is considered significant).

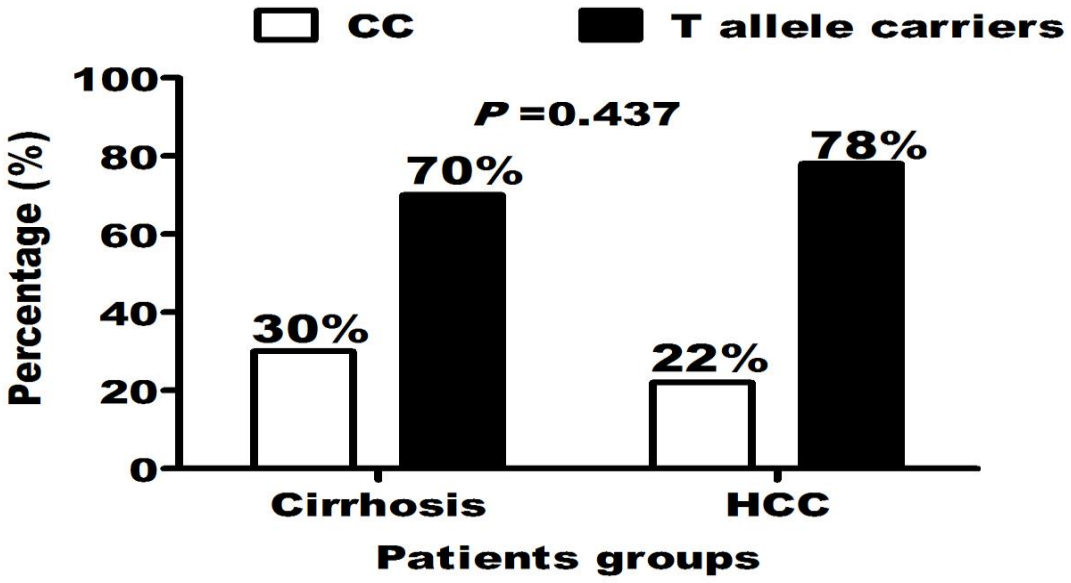

Figure 1. Distribution of IL-28B C/T alleles in cirrhosis and HCC. A chi-squared test (X^2) revealed that there was no significant difference in allele's distribution between patients. *P*>0.05 is considered non-significant.

IL-28B *rs12979860* and HCV-related HCC development

Concerning IL-28B genotypes distribution, T allele carriers (CT and TT combined) were slightly more frequent in HCC patients (39/50, 78%), without any significant difference (*P*=0.437), compared with patients with liver cirrhosis (21/30, 70%) (Figure 1). The role of IL-28B *rs12979860* SNP in prediction of HCC in CHC patients with liver cirrhosis was assessed by ROC curve analysis. As shown in Figure 2, there is no good reliable predictive power for IL-28B T allele to predict the HCC development (AUC=0.540).

Biochemical data among CC genotype and T allele carriers

Biochemical data that showed significant differences at *P* <0.05 level between HCC and cirrhotic patients did not show any significant differences when comparing patients with CC genotype with T allele carriers (Table 2). Spearman correlation analysis revealed that there was no association between IL-28B SNP and any of biochemical parameters (Table 3).

Table 2. Biochemical and virological parameters in relation with IL-28B genotypes

Parameter [a]	CC genotype	T allele carriers	*P* value
Number	20	60	–
Male/female	25/5	36/14	0.290
Age (years)	58.4 ± 1.5	55.7 ± 0.96	0157
AST (U/L)	65.9 ± 8.6	56.3 ± 3.6	0.232
ALT (U/L)	72.7 ± 11.6	57.4 ± 5.2	0.174
ALP (U /L)	86.9 ± 4.1	94.9 ± 3.0	0.164
Total bilirubin (mg/dL)	1.2 ± 0.15	1.3 ± 0.14	0.680
Direct bilirubin (mg/dL)	0.64 ± 0.21	0.66 ± 0.11	0.931
Albumin (g/dL)	3.8 ± 0.10	3.7 ± 0.07	0.397
Hemoglobin (g/dL)	13.8 ± 0.41	13.0 ± 0.25	0.148
WBCs ($\times10^3$/microliter)	5.1 ± 0.28	5.4 ± 0.29	0.486
RBCs ($\times10^6$/microliter)	4.6 ± 0.17	4.4 ± 0.08	0.235
Platelet count ($\times10^3$/microliter)	123.9 ± 11.5	119.9 ± 7.7	0.794
HCV-RNA ($\times10^5$ IU/ml)	5.0 ± 1.5	7.7 ± 1.2	0.253
Creatinine (mg/dL)	0.82 ± 0.03	0.86 ± 0.02	0.407
Fibroscan (kPa)	26.9 ± 2.23	24.5 ± 1.28	0.345

Variables were expressed as mean±SEM; [a] **Abbreviations**: AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; WBCs: white blood cells; RBCs: red blood cells. *P* <0.05 is considered significant

Table 3. Non-parametric correlations between IL-28B SNP and biochemical parameters

Factor correlated with IL-28B SNP	*r*	*P* value
Gender	0.051	0.654
Age	-0.127	0.261
AST	-0.113	0.320
ALT	-0.160	0.156
ALP	0.160	0.156
Total bilirubin	-0.001	0.991
Direct bilirubin	0.054	0.636
Albumin	-0.086	0.452
Hemoglobin	-0.156	0.166
WBCs	0.011	0.921
RBCs	-0.118	0.297
Platelet count	-0.063	0.582
HCV-RNA	0.131	0.246
Creatinine	0.110	0.332
Fibroscan	-0.162	0.151

Abbreviations: AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; WBCs: white blood cells; RBCs: red blood cells. *P* >0.05 is considered non-significant.

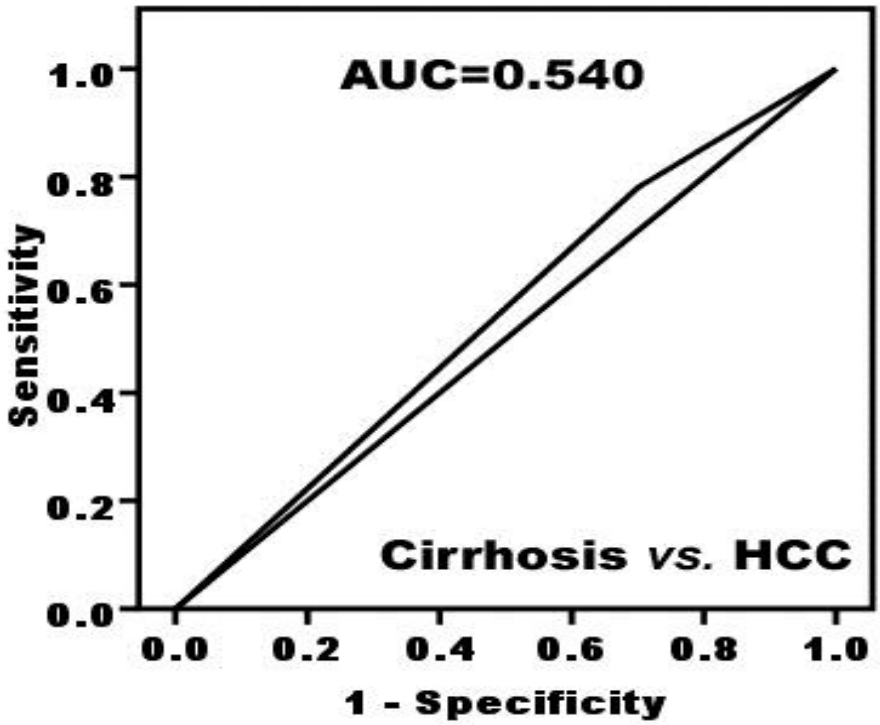

Figure 2. ROC curve analysis of IL-28B T allele for HCC prediction. AUC=1.0 is characteristic of an ideal test, whereas AUC≤0.5 indicates a test of no predictive value.

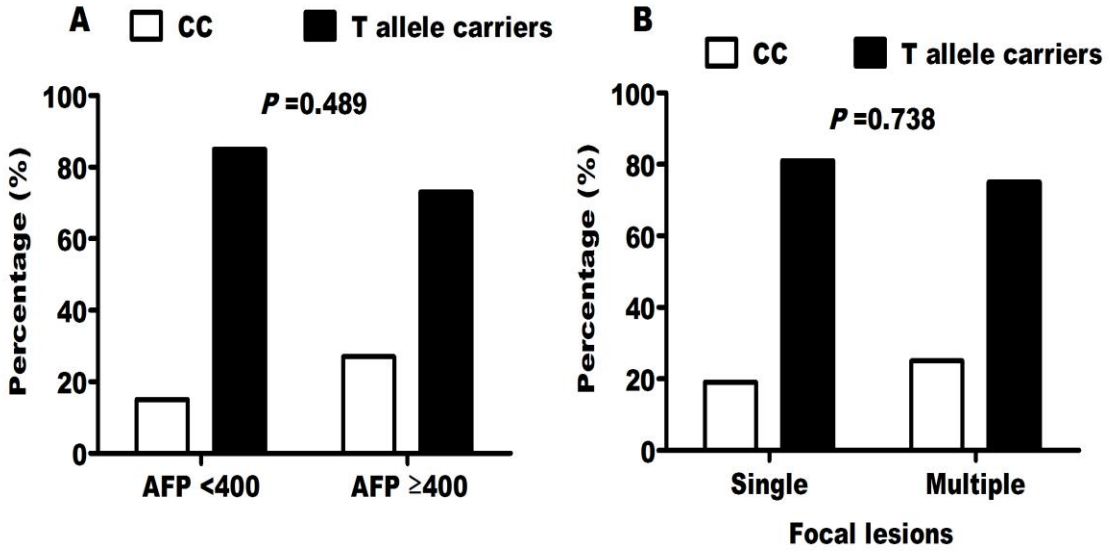

Figure 3. Distribution of IL-28B C/T alleles according to (A) AFP levels and (B) tumor focal lesions. A chi-squared test (X^2) revealed that there was no significant difference in alleles distribution between patients. *P*>0.05 is considered non-significant

IL-28B *rs12979860* SNP and some tumor features

Concerning the distribution of IL-28B *rs12979860* genotypes among low (<400 U/L) and high AFP levels (≥400 U/L), there was no significant difference (*P* =0.489) in CC genotype and T allele carriers distribution (Figure 3A). We used cutoff 400 U/L for AFP exactly as originally described (Bruix and Sherman, 2005). Moreover, in the term of number of lesions there was no significant difference (*P* =0.738) in CC genotype and T allele carriers distribution between patients with single and those with multiple tumors (Figure 3B).

DISCUSSION

The most frequent form of human genetic polymorphism are SNPs which are thought to be associated with patient response to drug treatment, population diversity and susceptibility to cancer (Shastry, 2002). It seems like a truth that IL28B *rs12979860* SNP may modify the treatment efficacy in CHC (Hodo et al., 2013). Also in patients with HCV infection, IL28B *rs12979860* is linked to disease progression and severity of hepatic fibrosis (Balagopal et al., 2010) and may be associated with a higher risk of HCC development (Fabris et al., 2011).

Some other studies investigated whether IL28B SNP involved in the development of HCV-induced HCC (Chang et al., 2015). However, the results are inconclusive and inconsistent. Some reported that IL-28B SNP appears to enhance the risk of HCC development in HCV patients with cirrhosis (Fabris et al., 2011) and others did not find this association (Agúndez et al., 2012). These studies have not been supported by other reports.

In the present study, the frequency of patients with CT/TT genotype did not differ between HCC and non-HCC (cirrhosis) groups, suggesting that the IL28B SNP may play little or no role in HCC development among Egyptian HCV patients. ROC curve analysis revealed that IL-28B *rs12979860* SNP have no role in HCC prediction among CHC patients with liver cirrhosis. These findings supported results of Joshita et al., who reported that IL28B SNP does not directly influence hepatocarcinogenesis in Japanese patients with chronic HCV infection (Joshita et al., 2012). Also, Agúndez et al., in Spain reported that there were no differences in the distribution of IL28B SNP genotypes between HCC and other chronic hepatitis C patients (Agundez et al., 2012). Other study in Spain found the same result in both HCV-related HCC and HCC induced from other etiologies (de la Fuente et al., 2017). Moreover, no significant association was found between IL28B *rs12979860* genotypes and the HCC risk in Turkish patients (Akkiz et al., 2014).

Conversely, in HCV patients who underwent liver transplantation (LT) Fabris et al., found that carriage of T allele seems to augment the risk of developing HCC (Fabris et al., 2011). In similar cohort of patients who underwent LT, Eurich et al., found that T allele may be regarded as a genetic risk factor for HCV-related carcinogenesis (Eurich et al., 2012). Also, other study reported the entire absence of the protective CC genotype in HCV patients with end stage liver disease (El-Awady et al., 2012). The discrepancy between our results and these studies may come from differences in patients analyzed (transplant or not), patients race, HCV genotypes, types of administered treatment.

Another important finding in this study was that there was no significant difference concerning the distribution of IL-28B *rs12979860* genotypes between low and high AFP levels and between patients with single and those with multiple tumors.

In summary, our findings showed that the IL-28B *rs12979860* genotype did not affect the development of HCC in Egyptian patients with chronic HCV infection. Other large scale, histological and functional studies were warranted to solve the discrepancy and clarify whether IL-28B SNP affect HCC development and if yes what the precise mechanism in hepatocarcinogensis.

CONCLUSION

In summary, our findings showed that the IL-28B *rs12979860* genotype did not affect the development of HCC in Egyptian patients with chronic HCV infection. Other large scale, histological and functional studies were warranted to solve the discrepancy and clarify whether IL-28B SNP affect HCC development and if yes what the precise mechanism in hepatocarcinogensis.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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REFERENCES

Agúndez JA, García-Martin E, Maestro ML, et al., 2012. Relation of IL28B gene polymorphism with biochemical and histological features in hepatitis C virus-induced liver disease. PLoS One; 7: e37998.

Agundez JA, Garcia-Martin E, Maestro ML, et al., 2012. Relation of IL28B gene polymorphism with biochemical and histological features in hepatitis C virus-induced liver disease. PloS one; 7: e37998.

Akkiz H, Kuran S, Akgollu E, Uskudar O, Bekar A, Bayram S, 2014. The role of interleukin 28B gene polymorphism in Turkish patients with hepatocellular carcinoma. Annals of hepatology; 13: 788-795.

Attallah AM, El-Far M, Omran MM, et al., 2016. GPC-HCC model: a combination of glybican-3 with other routine parameters improves the diagnostic efficacy in hepatocellular carcinoma. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine; 37: 12571-12577.

Balagopal A, Thomas DL, Thio CL, 2010. IL28B and the control of hepatitis C virus infection. Gastroenterology; 139: 1865-1876.

Bruix J, Sherman M, 2005. Management of hepatocellular carcinoma. Hepatology (Baltimore, Md); 42: 1208-1236.

Chang KC, Tseng PL, Wu YY, et al., 2015. A polymorphism in interferon L3 is an independent risk factor for development of hepatocellular carcinoma after treatment of hepatitis C virus infection. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association; 13: 1017-1024.

de la Fuente S, Citores MJ, Duca A, et al., 2017. Interleukin-28B TT genotype is frequently found in patients with hepatitis C virus cirrhosis but does not influence hepatocarcinogenesis. Clinical and experimental medicine; 17: 217-223.

El-Awady MK, Mostafa L, Tabll AA, et al., 2012. Association of IL28B SNP with Progression of Egyptian HCV Genotype 4 Patients to End Stage Liver Disease. Hepatitis Monthly; 12: 271-277.

Eurich D, Boas-Knoop S, Bahra M, et al., 2012. Role of IL28B polymorphism in the development of hepatitis C virus-induced hepatocellular carcinoma, graft fibrosis, and post-transplant antiviral therapy. Transplantation; 93: 644-649.

Fabris C, Falleti E, Cussigh A, et al., 2011. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. Journal of hepatology; 54: 716-722.

Hodo Y, Honda M, Tanaka A, et al., 2013. Association of < em> Interleukin-28B Genotype and Hepatocellular Carcinoma Recurrence in Patients with Chronic Hepatitis C. Clinical Cancer Research; 19: 1827.

Joshita S, Umemura T, Katsuyama Y, et al., 2012. Association of IL28B gene polymorphism with development of hepatocellular carcinoma in Japanese patients with chronic hepatitis C virus infection. Human immunology; 73: 298-300.

Kitson MT, George J, Dore GJ, et al., 2014. Interleukin-28B rs12979860 C allele: Protective against advanced fibrosis in chronic hepatitis C genotype 1 infection. Journal of gastroenterology and hepatology; 29: 1458-1462.

Knapp S, Warshow U, Ho KMA, et al., 2011. A Polymorphism in IL28B Distinguishes Exposed, Uninfected Individuals From Spontaneous Resolvers of HCV Infection. Gastroenterology; 141: 320-325.e322.

Sandrin L, Fourquet B, Hasquenoph JM, et al., 2003. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound in medicine & biology; 29: 1705-1713.

Shastry BS, 2002. SNP alleles in human disease and evolution. Journal of human genetics; 47: 561-566.

Thomas DL, Thio CL, Martin MP, et al., 2009. Genetic variation in IL28B and spontaneous

clearance of hepatitis C virus. Nature; 461: 798-801.

Ziada DH, El Sadany S, Soliman H, et al., 2016. Prevalence of hepatocellular carcinoma in chronic hepatitis C patients in Mid Delta, Egypt: A single center study. Journal of the Egyptian National Cancer Institute; 28: 257-262.