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Association of Genetic Variation in IL28B rs12979860 with Development of Hepatocellular Carcinoma

Abu Saleh Mohammad Sadequl Islam[1], Mamun Al Mahtab[1], Ayub Al Mamun[1], Mohammad Fazal Karim[2], Sheikh Mohammad Fazle Akbar[3], Mohammad Eunus Ali[4], Humayun Sattar[4], Salimur Rahman[1]

[1]Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka 1000, Bangladesh
[2]Department of Hepatology, Sir Salimullah Medical College, Dhaka 1100, Bangladesh
[3]Deartment of Medical Sciences, Toshiba General Hospital, Tokyo 140-8522, Japan
[4]Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka 1000, Bangladesh

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***Corresponding author:** Mamun Al-Mahtab, Department of Hepatology, Bangabandhu Sheikh Mujib Medical Unievsrity, Shahbagh, Dhaka 1000, Bangladesh, Phone: +880-171-1567275; Fax: +880-2-8826840; E-mail: shwapnil@agni.com*

Abstract

The highest incidence of hepatocellular carcinoma (HCC) is in Asia, accounting for about 76% of all cases worldwide. In South East Asia, hepatitis B is the most common underlying cause. Although numerous associations between environmental factors (e.g. cigarette smoking, heavy alcohol drinking, male gender, older age), viral factors (e.g. viral load, active replication, genotype, core promoter mutations) and the development of HCC in chronic HBV infection have been identified, a clear understanding of the role of host genetics remains elusive. Bangladesh is a densely populated country with about 160 million populations, where HBsAg positivity in the healthy population is 5.4%. To evaluate the role of host IL28B (interleukin 28B; interferon lambda 3) single nucleotide polymorphism (SNP) in predicting hepatitis B virus (HBV)-related HCC susceptibility. Single SNP in the IL28B gene (rs12979860C/T) were examined in 116 subjects (including 44 HBV-related HCC patients, 42 non-HCC patients with chronic hepatitis B and 30 healthy controls). The study was done at the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from January 2012 to December 2013.The polymorphism of IL28B rs12979860 was analyzed by a genotyping technique, based on polymerase chain reaction (PCR) followed by restriction enzyme analysis at the Department of Microbiology & Immunology, BSMMU. The frequency of CC homozygosity was 70% in healthy controls and 45.5% in HCC, the difference being statistically significant ($\chi2=4.35$, $P=0.03$). Statistically significant difference was also seen between non-HCC patients with chronic hepatitis B (CHB) (69%) and HCC (45.5%) ($\chi2=4.35$, $P=0.03$). However, this significant finding was not seen between non-HCC patients with chronic hepatitis B (CHB) and healthy controls. Carriers of the minor T allele in rs12979860 had a higher risk of HCC compared with non-carriers ($\chi2=4.78$, $P=0.02$). Our results suggest that, T allele and non-CC genotypes have strong predictive effect of developing HCC and IL28Brs 12979860 C/T polymorphism might influence susceptibility to HCC.

Keywords: Hepatitis B v irus, hepatocellular carcinoma; Interleukin; Genetic polymorphism; Interferon lambda (λ)

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor and the third most common cause of cancer deaths worldwide [1]. The etiological agent of HCC is known in more than 90% of cases. In South East Asia, hepatitis B is the most common underlying cause. The highest incidence of HCC is in Asia, accounting for about 76% of all cases worldwide [2].

HBV infection is a serious global health problem. About 378 million people throughout the world are chronically infected with this virus [3]. Approximately 15-40% of chronic hepatitis B (CHB) patients will develop cirrhosis, liver failure and HCC [4]. Bangladesh is within the intermediate zone of prevalence of HBV infection. HBsAg positivity in healthy population is 5.4% [5].

With antiviral, immune modulatory and perhaps antitumor activities, standard or pegylated interferon-alpha (IFN-α) is the current therapeutic option of choice for patients with CHB. Strong evidence from prospective cohort studies suggest that IFN-α treatment, by suppressing HBV replication, decreases overall HCC incidence, with a more marked effect in sustained responders [6]. However, the putative beneficial effect of IFN treatment is difficult to prove due to the wide variability of individual outcomes in the natural course of these diseases, in which genetic factors are likely to play a role [7].

As a therapeutic agent, IFN- λ might have longer and more potent effects than IFN-α. IFN- λ interacts with a trans-membrane receptor to induce potent antiviral responses that are mediated through the activation of the JAK-STAT and MAPK pathways [8]. In vitro and in vivo models have shown the importance of IFN-λ in the immune response to several viral pathogens, including hepatitis C virus (HCV) and HBV [9].

Several genome-wide association studies (GWAS) have identified a strong association between single nucleotide polymorphisms (SNPs) in and near IL28B, which encodes IFN-

λ and response to therapy [10] and with spontaneous HCV clearance [11]. It is possible that similar effects occur in patients with chronic HBV infection, since IFN- λ inhibits HBV and HCV replication in an experimental model [12].

Based on this hypothesis, we evaluated whether specific IL28B rs12979860 is associated with risk of developing HBV-related HCC, because IL28B rs12979860 polymorphic alleles affect responses to IFN, and IFN therapy changes the prognosis of CHB.

Methods

Study Population

This is a hospital based case-control study of 116 subjects, including 44 HBV-related HCC patients, 42 non-HCC patients with CHB and 30 healthy controls. They attended the Out-patient and In-patient Departments of Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from January 2012 to December 2013. Aims and objectives of the study along with its procedure, risks and benefits of this study was explained to the subjects and attendants in easily understandable local language (Bangla) and then informed written consent was taken from each participant. Prior to the commencement of the study, the research protocol was approved by the Institutional Review Board of BSMMU.

Patients were divided into three groups. Group A (HBV-related HCC patients), Group B (non-HCC patients with CHB) and Group C (healthy controls). The inclusion criteria were (1) in group A, HCC patients were recruited prospectively. The diagnosis of HCC was confirmed by α-fetoprotein elevation (> 400 ng/ml) combined with computed tomography (CT) and/or magnetic resonance imaging (MRI) and cytology; (2) for group A and B, HBsAg (hepatitis B virus surface antigen) positivity, anti-HBc total (hepatitis B virus core antibody total) positivity and HBeAg (hepatitis B virus e antigen) or HBeAb (hepatitis B virus e antibody) positivity for >6 months; and (3) for Group C, HBsAg negativity. Exclusion criteria were, alcohol abuse (>20g/day), infection with HCV (anti-HCV positivity), autoimmune hepatitis, Wilson`s disease, haemochromatosis and age < 18 years.

DNA extraction and genotyping

IL28B rs12979860 genotyping was done at the Department of Microbiology and Immunology, BSMMU. A single sample of approximately 3-4 ml of venous blood was collected from each participant into EDTA-containing blood tubes. Genomic DNA was extracted from a leukocyte pellet using 20% chelex solution followed by heatdenaturation of cell debris. The SNP in IL28B rs12979860C/T, selected for the present study are recorded in the public dbSNP database. Approximate 30ng extracted DNA was amplified with allele specific primer using ABI 2720 system (Applied Biosystems, Foster City, CA). Primers for PCR-RFLP of rs12979860 polymorphism were designed using Primer-Blast, considering the homology in the region IL28B gene. PCR was performed using Master buffer® PCR (Texas Biogeneinc, USA) with a 20 µl reaction tube type. Briefly, 10-30 ng of genomic DNA was amplified with 10 pmol of rs12-F and rs12-R primers for the genomic region containing rs12979860. The PCR temperature profile comprised 94°C for 5 min; 35 cycles of 94°C for 20 sec, 66°C for 20 sec, 72°C for 20 sec and 72°C for 5 min, according to the manufacturer's instructions. For RFLP analysis, the PCR product of rs12979860 was digested with 10 units of Bsh1236I (BstUI) restriction endonuclease (Fermentas, Vilnius, Lithuania) for one hour. The digestion products were separated on a 3% agarose gel alongside the Gene aid TM 100 bp DNA Ladder. In each PCR-RFLP genotyping run, as a control for the enzymatic activity of Bsh1236I rs12979860 CC DNA was included. Genotyping was performed without knowing the subject's case or control status and more than 10% of samples were randomly selected for repeat analysis.

Procedure for fine needle aspiration (FNA) from liver space occupying lesions SOL(s)

After taking informed written consent, patients laid with empty bladder. The site was painted with iodine solution and draped. Skin and deeper tissue was infiltrated with local anesthesia (2% xylocaine) at the proposed puncture site using a 23 G needle. Under real-time USG guidance and using 22 G disposable spinal needles the cavity was entered and aspirated material was collected. The prepared glass slides ware fixed with 95% ethanol and kept in Kaplan's jar after labeling. Samples were sent for cytopathological examination to the Department of Pathology, BSMMU. Dressings were applied at the needle puncture sites and patients were followed up for next 6 hours.

Statistical Analysis

All data was recorded systematically in a preformed data collection sheet and quantitative data expressed as mean ± SD. Qualitative data analyzed by chi square test and quantitative data by student's T test or Mann Whitney's U test. Differences in laboratory parameters compared using one-way ANOVA. The genotype distribution of rs12979860 were in Hardy-Weinberg equilibrium and analyzed by the chi square test. P value of ≤ 0.05 was considered to be statistically significant. All statistical computations were performed by using SPSS version 20 (Statistical Package for Social Science).

Results

Demographic and laboratory characteristics

Table 1 includes the demographic and laboratory parameters for cases within different disease groups and healthy controls, including gender, age, serum albumin, serum bilirubin, alanineaminotransferase, α-fetoprotein, haemoglobin, prothombin time, platelet count and IL 28B genotype. HBV-related HCC patients were older than patients in the other two groups and had a higher proportion of males. The HBV-related HCC group and the other two groups without HCC (healthy controls, non-HCC patients with CHB) had significantly different laboratory results for serum albumin, serum bilirubin, α-fetoprotein and prothombin time ($p < 0.05$).

Genotype distribution

Genotype distribution analysis showed that the studied SNPs

Table 1: Demographic and laboratory parameters of the subjects in the study.

Groups	HBV- related HCC (n = 44)	Non-HCC patients with CHB (n = 42)	Healthy controls (n = 30)
Demographic parameters			
Gender (M/F)	40/4	38/4	10/20
Age (Y) (χ ± SD)	48.20 ± 12.91	37.69 ± 13.74	46 ± 11.44
History of smoking	26/18	13/29	0/30
Presence of cirrhosis	35/9	17/25	0/30
Laboratory parameters (χ ± SD)			
T-Bil (μmol/l)	94.98 ± 115.06	64.90 ± 120.05	11.37 ± 3.7
ALB (g/dl)	3.02 ± 0.55	3.19 ± 0.70	3.90 ± 0.24
ALT (U/l)	76.52 ± 51	123.31 ± 302.33	35.50 ± 7.63
AFP (ng/ml)	15242.37 ± 1798	15.71 ± 66.52	4.31 ± 2.943
Hb (g/dl)	11.43 ± 1.72	12.73 ± 2.53	12.47 ± 0.96
PT (sec)	15.12 ± 2.20	14.95 ± 3.27	12.72 ± .87
INR	1.28 ± .19	1.126 ± 0.28	1.06 ± 0.08
PLT (109/L)	227.2 ± 102.71	209.88 ± 88.18	298.83 ± 71.41
IL 28B Genotype (rs12979860)			
CC/Non CC	20/24	29/13	21/9

Table 2: Genotype distribution and allele frequencies of SNP in IL28 rs12979860 gene.

SNPs ID (gene)	**Genotype n (%)**				**Allele n (%)**
rs12979860 C/T	TT	TC	CC	T	C
HBV- related HCC	7(15.9)	17(38.6)	20(45.5)	31(35.2)	57(64.8)
Hepatitis B	3(7.1)	10(23.8)	29(69)	16(19)	68(81)
Control	0(0)	9(30)	21(70)	9(15)	51(85)
Total	10(8.6)	36(31)	70(60.3)	56(24.14)	176(75.86)

were in Hardy-Weinberg equilibrium. No departure from the Hardy-Weinberg distribution was observed for each genotype (p value never significant) in control subjects and in patients with HCC. No significant SNP-specific deviation ($p < 0.05$) was observed.

Associations of IL28B polymorphism and development of hepatitis B-related hepatocellular carcinoma

Table 2 shows comparison of allele frequencies and genotype distributions of SNPs among HCC, non-HCC patients with CHB and healthy controls. There were significant differences among HBV-related HCC, non-HCC patients with CHB and healthy controls groups ($\chi2=9.547$, $p=0.04$). Notably, the frequency of CC homozygosity was 70% in healthy controls and 45.5% in HCC, the difference being significant ($\chi2=4.35$, $p=0.037$). And then, the frequency of CC was 45.5% in HCC and 69% in non-HCC patients with CHB, the difference being significant ($\chi2=4.35$, $p=0.037$). However, this significant finding was not seen between non-HCC patients with CHB and healthy controls ($\chi2=0.007$, $p=0.931$). In the analyses by genotypes, TT homozygous and CT heterozygous patients both had a higher risk of developing HBV-related HCC compared to patients carrying the common genotype (CC). Minor T allele carriers had a higher risk of CHB and HCC than healthy controls ($\chi2=12.6$, $p=0.002$) and the T allele was therefore defined as the risk allele.

Discussion

This case control study was carried out at the Department of Hepatology. We analyzed the genetic variation IL28B rs12979860 in HBV related HCC patients, non-HCC with CHB patients and healthy control. We investigated 116 patients without history of significant alcohol intake (> 20gm/day), co-infection with HCV, or age < 18 year. 86 CHB patients with serum HBsAg positivity and 30 healthy individuals having serum HBsAg negativity were enrolled as controls.

The significance of SNPs in cancer is a recent finding. There are substantial publications in the literature concerning genetic variation with various types of malignancy in humans, for example EGF gene and malignant melanoma [13], EGF gene and glioblastoma multiform patients [14], CXCL12 polymorphism

with acute myeloid leukemia [15], CYP1A1 T3801C polymorphism and cervical neoplasia risk [16], MDM2 T309G and prostate cancer [17].

HBV infection accounts for most primary HCC, and treating HBV infection substantially reduces the risk of HCC development, as the viral load is found to be the most important factor leading to cirrhosis and cancer development in the liver [18, 19]. Although chronic HBV infection is recognized as the most important causal factor for HCC in humans, some HCC cases are without chronic HBV infection, suggesting the presence of important co-factors in HBV-related HCC.

Different approaches have been used to identify genetic susceptibility factors for the natural course and treatment response in HCV and HBV. Recently, allelic variants in the IL28B gene have gained major interest as panel of SNPs were identified to be strongly associated with treatment-induced and spontaneous clearance of HCV. The associated region (19q13) encodes 3 cytokine genes (IL28A, IL28B and IL29) that belongs to the IFN- λ (also named type III IFN) family. IFN λs interact with a trans-membrane receptor to induce potent antiviral responses [20-22]. This antiviral activity is mediated through the activation of the JAK-STAT (IFN-αs, IFN-γs and IFN-λs) and MAPK (IFN- αs and IFN- λs) pathways. In vitro and in vivo models have shown the importance of IFN- λs in the immune response and in the up-regulation of transcription of IFN-stimulated genes (ISGs) that are required to control viral infection, including herpes simplex virus [23], HIV [24], HBV [12] and HCV [26]. IFN-λ seems to inhibit HBV and HCV replication in experimental model [26]. Therefore, in studies aimed at assessing the role of IL28B rs12979860C/T polymorphism in patients with chronic HCV infection, similar effects could also be expected in patients with chronic HBV infection.

We observed a trend for a relationship between rs12979860in HBV-related HCC susceptibility. Furthermore, the frequency of the TC+TT genotype was significantly increased in HBV-related HCC patients compared to healthy individuals and CHB patients, while both C allele and CC genotype frequencies of healthy controls were protective, which indicates that the rs12979860 T/C polymorphism is associated with the carcinogenic process of HBV-related HCC. However, a significant difference was only identified in subjects between HBV-related HCC and non-HCC individuals (including CHB and healthy controls). This may be due to the small sample size of non-HCC patients with CHB. The protective effect of the C allele in our study is consistent with the results the study by another group [26], which identified a SNP (C/T; rs12979860) 3 kb upstream of IL28B that was associated with a favorable treatment response in patients with HCV genotype 1 infection. C allele at rs12979860 was already known to enhance the inhibitory effect on JAK-STAT and on ISGs activation of IFN-λ, the T/C variant was hypothesized to decrease the HBV clearance capability of the immune response and to up-regulate transcription of antiviral proteins.

It has been pointed out in a study that the IL28B rs12979860 C/T polymorphism might affect susceptibility to CHB and progression of HCC [27]. Of note, the T allele and non-CC genotypes have strong predictive effect of increasing susceptibility of CHB and HCC. They examined in 330 subjects (including 154 HBV-related HCC patients, 86 non-HCC patients with CHB, 43 HBV self-limited infections and 47 healthy controls) and significant difference was seen between healthy controls and CHB (HBV-related HCC patients, non-HCC patients with CHB) (p=0.01), but not between HBV self-limited and healthy controls. Carriers of the minor T allele in rs12979860 had a higher risk of HCC compared with non-carriers (p=0.04). Nevertheless, our study, which attempted to link these genetic components to HCC susceptibility, was limited due to its cohort-specific nature. GWAS have been utilized to perform large-scale interrogation of genetic variants in cancer.

Conclusion

We report that rs12979860 in the IL28B gene might be a risk factor for developing HBV-related HCC. Screening of these polymorphisms and functional studies would be useful to clinical practice for identifying groups at high risk of HCC and might help to modify the design of HCC surveillance for patients with chronic HBV infection.

References

1. Jemal A, Bray F, Center MM, Ferly J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(1):69-90. doi: 10.3322/caac.20107.
2. Bosch FX, Ribes J, Cléries R, Diaz M. Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2005;9(2):191–211.
3. Franco E, Bagnato B, Maria MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: Epidemiology and prevention in developing countries. World J Hepatology. 2012;4(3):74-80. doi: 10.4254/wjh.v4.i3.74.
4. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: Summary of a Clinical Research Workshop. Hepatology. 2007;45:1056-1075.
5. Mahtab MA, Rahman S, Foster G, Khan M, Karim MF, Solaiman S, et al. Epidemiology of Hepatitis B Virus in Bangladeshi General Population. Hepatobiliary Pancreat Dis Int. 2008;7(6):595-600.
6. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130(3):678–686.
7. Thio CL, Thomas DL, Carrington M. Chronic viral hepatitis and the human genome. Hepatology. 2000;31(4):819–827.
8. Abushahba W, Balan M, Castaneda I, Yuan Y, Reuhl K, Raveche E, et al. Antitumor activity of Type I and Type III interferons in BNL hepatoma model. Cancer Immunol Immunother. 2010;59:1059-1071. doi: 10.1007/s00262-010-0831-3
9. Hong SH, Cho O, Kim K, Shin HJ, Kotenko SV, Park S. Effect of interferon-lambda on replication of hepatitis B virus in human hepatoma cells. Virus Res. 2007;126(1-2):245–249. doi:10.1016/j.virusres.2007.03.006
10. Thomas DL, Thio CL, Maureen MP, Qi Y, Ge D, Uigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461(7265):798-801. doi: 10.1038/nature08463.
11. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28Bwith response to pegylated

interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genetics. 2009;41(10):1105–1109. doi: 10.1038/ng.449. Epub 2009 Sep 13.

12. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. J Virol. 2005;79(6):3851–3854.

13. Shahbazi M, Pravica V, Nasreen N, Fakhoury H, Fryer AA, Strange RC, et al. Association between functional polymorphism in EGF gene and malignant melanoma. Lancet. 2002;359(9304):397–401.

14. Bhowmick DA, Zhuang Z, Wait SD, Weil RJ. A functional polymorphism in the EGF gene is found with increased frequency in glioblastoma multiforme patients and is associated with more aggressive disease. Cancer Res. 2004;64(4):1220-1223.

15. Dommange F, Cartron G, Espanel C, Gallay N, Domenech J, Benboubker L, et al. CXCL12 polymorphism and malignant cell dissemination/ tissue infiltration in acute myeloid leukemia. FASEB J. 2006;20(11):1913-1915.

16. Liangbin X, Jing G, Yan L, Ke Wu. Significant association between CYP1A1 T3801Cpolymorphism and cervical neoplasia risk: a systematic review and meta-analysis. Tumor Biol. 2013;34(1):223–230. doi: 10.1007/s13277-012-0542-9

17. Yang j, Gao W, Song NH, Wang W, Zhang JX, Lu P, et al. The risks, degree of malignancy and clinical progression of prostate cancer associated with the MDM2 T309G polymorphism: a meta-analysis. Asian J Androl. 2012;14(5):726–731. doi: 10.1038/aja.2012.65.

18. Chen CJ, Yang HI, Su J, Jen C, You SN, Lu SN, et al. Risk of Hepatocellular Carcinoma Across a Biological Gradient of Serum Hepatitis B Virus DNA Level. JAMA. 2006;295(1):65–73.

19. Yu MC, Yuan JM, Govindrarajan S, Ross RK. Epidemiology of hepatocellular carcinoma. Canadian J Gastroenterol. 2000;14(8):703–709.

20. Li W, Lewis-Antes A, Huang J, Balan M, Kotenko SV. Cell Prolif. 2008;41(6):960-979. DOI: 10.1111/j.1365-2184.2008.00558.x

21. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiol Rev. 2006;28(1):112-125. doi: 10.1093/epirev/mxj009.

22. Li M, Liu X, Zhou Y, Su BB. Interferon-lambdas: the modulators of antivirus, antitumor, and immune responses. J Leukoc Biol. 2009;86(1):23–32. doi: 10.1189/jlb.1208761

23. Melchjorsen J, Siren J, Julkunen I, Paludan SR, Matikainen S. Induction of cytokine expression by herpes simplex virus in human monocyte-derived macrophages and dendritic cells is dependent on virus replication and is counteracted by ICP27 targeting NF-kappaB and IRF-3. J General Virol. 2006;87(Pt5):1099–1108.

24. Hou W, Wang X, Ye L, Zhou L, Yang ZQ, Riedel E, et al. Lambda interferon inhibits human immunodeficiency virus type 1 infection of macrophages. J Virol. 2009;83(8):3834-3842. doi: 10.1128/JVI.01773-08

25. Hong SH, Cho O, Kim K, Shin HJ, Kotenko SV, Park S. Effect of interferon-lambda on replication of hepatitis B virus in human hepatoma cells. Virus Resch. 2007;126(1-2):245–249. doi:10.1016/j.virusres.2007.03.006

26. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399-401. doi:10.1038/nature08309.

27. Ren S, Lu J, Du X, Huang Y,Ma L, Huo H, Chen X, Wei L. Genetic variation in IL28B is associated with the development of hepatitis B-related hepatocellular carcinoma. Cancer Immunol Immunother. 2012;61(9):1433–1439. doi: 10.1007/s00262-012-1203-y