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Role of TLL1 Gene Polymorphism in Development of Hepatocellular Carcinoma in Egyptian Cirrhotic Patients Achieving Sustained Virological Response by Direct Acting Antiviral Agents for HCV

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Abstract

Background and aim: Host genetic factors have been recognized as potential determinants of denovo occurrence of hepatocellular carcinoma (HCC) in patients with HCV related cirrhosis even after achieving sustained virological response (SVR) with direct antiviral agents (DAAs). We investigated whether rs17047200 in TLL1 gene is associated with early development of HCC in Hepatitis C Virus (HCV) cirrhotic Egyptian patients treated after eradication with antivirals (DAAs)

Materials and Methods: 50 patients with SVR developed denovo HCC (HCC group) within 6 months after end of treatment were included and compared to 50 patients who achieved SVR without HCC development (non HCC group). TLL1 rs17047200 polymorphism was analyzed in both groups.

Results: In HCC group rs17047200 in TLL1 gene was AA in 34 (68.0%), AT in 9 (18%), TT in

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2 (4%) and undetermined in 5 patients (10%). In non HCC group, AA genotype was in 25 (50.0%), AT in 16 (32 %), TT in 2 (4%) and undetermined in 7(14%), with no significant difference between 2 groups. There was no significant association between TLL1 gene variants and HCC features or baseline clinical and investigational characteristics in HCC group. Multivariate analyses identified age >50, male sex, baseline higher liver stiffness and higher baseline FIB-4 score as independent predictors of HCC occurrence in patients achieving SVR within 6 months after DAAs for HCV infection.

Conclusion: TLL1 genotypes do not predict HCC development after HCV eradication by DAAs.

Keywords: sustained virological response, Antiviral Agents, Carcinoma, Hepatocellular, Tolloid-Like Metalloproteinases

Introduction

Achieving a sustained viral response (SVR) by direct-acting antiviral drugs (DAAs) has been shown to improve survival in cirrhotic patients with hepatitis C virus-infection. (1, 2). Nevertheless, the risk of development of denovo hepatocellular carcinoma (HCC) in cirrhotic patients after SVR still present and necessitates continuing long-life surveillance (3, 4). In addition, it has been suggested that HCC may early occur or reoccur within 6 months after SVR by DAAs therapy (5). Because this phenomenon was not seen in patients treated with interferon or ribavirin, some experts speculate that these novel DAAs may in fact play a significant role in tumor development (6) However, data on HCC risk following DAAs are still sparse and conflicting(7).

From this perspective, the need to identify possible HCC predictors in this group of patients assumed great importance, to tailor individualized risk profiles that would allow for surveillance and treatment planes. Among these predictors are host genetic factors. Conflicting results have been published in western and eastern population regarding relationship between TLL1 variant and HCC development in patients treated and cured with DAAs (8, 9).

Therefore, our aim was to investigate the relationship between TLL1 polymorphism and the risk of early denovo HCC development in Egyptian patients with HCV related cirrhosis who achieved SVR with DAA.

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Materials and Methods

This cohort study was done on 100 cirrhotic patients after eradication of hepatitis C virus (HCV) by DAAs during the interval between January 2017 and December 2019. From 5760 patients fulfilling the inclusion and exclusion criteria, 50 (0.68%) patients developed HCC (HCC group) within 6 months after treatment and another 50 patients without HCC development (non HCC group) were enrolled in the study. Patients with etiology of liver disease other than HCV, previous history of HCC, current HCC, atypical hepatic focal lesions and with extra hepatic tumors at baseline were excluded.

All included patients received DAAs according to National Committee for Control of Viral Hepatitis in Egypt (NCCVH). Patients received sofosbuvir/ daclatasvir (SOF/DACLA) or sofosbuvir/ daclatasvir and ribavirin (SOF/DACLA/RIBA) for 12 weeks. SVR was confirmed by undetectable HCV Polymerase Chain Reaction (PCR) 3 month after end of treatment. Liver cirrhosis before treatment was confirmed clinically, biochemically, by presence of ultrasound features of liver cirrhosis and by using noninvasive tools as transient elastography (F3: 9.5-12.4 kpa is severe fibrosis and F4: >12.5 kpa is cirrhosis) and FIB-4 index > 3.25. HCC diagnosis was established according to the international guidelines (liver nodule > 1 cm with enhancement in the early arterial phase and washout in portovenous and delayed phases compared to the rest of the liver detected by dynamic imaging as computed tomography (CT) and/or Magnetic Resonance Imaging (MRI)).

Before starting DAAs, all enrolled patient were subjected to full history taking and clinical examination. In addition, full blood count (FBC), Alanine aminotransferase, Aspartate aminotransferase, serum total bilirubin, serum albumin and international normalized ratio (INR), serum creatinine and alpha-fetoprotein (AFP) were done at baseline, every month during treatment, at end of treatment, 3 and 6 months after treatment. Real time quantitative HCV PCR was done at baseline, end of treatment and 3 months after treatment. Abdominal ultrasound was done every 3 months before and at end of treatment, 3 and 6 months after treatment. Transient elastography was done before treatment. Triphasic CT and/or MRI abdomen was done in suspected cases of HCC to confirm the diagnosis.

All enrolled patients were tested for Nucleotide polymorphism in rs17047200 in TLL1 gene. Samples were collected at 6 months after end of treatment from patients who didn't

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develop HCC and at the time of HCC diagnosis from those who developed HCC. Whole blood was collected from all patients and centrifuged to separate the buffy coat. Genome Deoxyribonucleic acid (DNA) was extracted from the buffy coat using a QiA Amp DNA blood mini kit (Qlagen, Germany). DNA extraction took place in biosafety cabinets level II (lobanco, USA) according to the manufactures instructions. A7500 Fast real-time PCR system (Applied biosystems, Foster City CA. USA) used for Genetic polymorphism of SNPs genotyping

The collected data was revised, coded, tabulated and introduced to a computer using Statistical package for Social Science (SPSS 23). Data was presented as mean, Standard deviation (± SD) and range for parametric numerical data; median and interquartile range (IQR) for non-parametric numerical data and frequency and percentage of non-numerical data. Student T Test was used to assess the statistical significance of the difference between two study group means. Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. One Way ANOVA test and Kruskal Wallis test were used to assess the statistical significance of the difference of a quantitative variable between more than two study groups. Chi-Square test and Fisher's exact test were used to examine the relationship between two qualitative variables. Logistic regression analysis was performed to evaluate the independent factors associated with HCC development after DAA. All statistical analyses were based on two-tailed hypothesis tests with a significance level of $p < 0.05$

Results:

This study included 100 patients: 59 patients were males and 41 patients were females and the mean age was 56.42±10.45 years. Diabetes mellitus was present in 63 patients. Only 1 patient was child class B and the rest of patients were Child class A. Seventy four patients were given SOF/DACLA and 26 were given SOF/DCLA/RIBA. Baseline demographic and investigational data of the 2 groups were compared in table1. The mean age (58.98 vs 54.02 years) and model of end stage liver disease (MELD) score (6.40 vs 5.48); median international normalized ratio (INR) (1.21 vs 1.1), total bilirubin (0.8 vs 0.6 mg/dl), FIB-4 (4.2 vs 3.66) and liver stiffness (19.5 vs 15 kPa) and percentage of male gender (74% vs 44%) and diabetes (74% vs 32%) in HCC group were significantly higher than that in Non HCC group.

Rs17047200 in TLL1 gene variants was AA in 59%, AT in 25%, TT in 4% and undetermined in 12% of all enrolled patients. Rs17047200 in TLL1 gene variants were identified

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and compared in both groups in table 2. In HCC group Rs17047200 in TLL1 gene variants was AA in 34 (68.0%), AT in 9 (18 %) and TT in 2 (4%) and undetermined in 5 patients (10%). In non HCC group Rs17047200 in TLL1 gene variants was AA in 25 (50.0%), AT in 16 (32 %) and TT in 2 (4%) and undetermined in 7(14%). There was no significant difference between 2 groups regarding gene variants distribution.

Fifty patients had denovo HCC within 6 months after SVR, 6 (12%) patients developed portal vein thrombosis, 11 (22%) patients had lesions > 3cm, 3 patients had extrahepatic spread and 9 patients had multinodular lesions. There was no significant association found between rs17047200 in TLL1 gene variants and any of those HCC characteristics of studied population in HCC group. (Table 3)

In HCC group, the frequency of presence TLL1 rs17047200 homogenous (AA+TT), heterogeneous (AT/TT) and undetermined variants was calculated in patients and compared regarding baseline clinical and investigational features (table 4). When compared, there were no significant differences and no significant associations found between rs17047200 in TLL1 gene variants and any of the studies parameters

Age >50 (OR: 6.517; 95% CI: 2.211- 19.215), male (OR: 3.622; 95% CI: 1.559- 8.418), patients with diabetes (OR: 6.048; 95% CI: 2.540- 14.339), baseline total bilirubin >1 (OR: 5.062; 95% CI: 1.703- 10.050), INR>1.18 (OR: 7.333; 95% CI: 2.652- 20.282), MELD> 5.72 (OR: 2.901; 95% CI: 1.288- 6.534), higher liver stiffness (OR: 4.373; 95% CI: 1.854-10.316) and higher FIB-4 (OR: 4.125; 95% CI: 1.611-10.559) were identified as risk factors for HCC development in univariate analysis. On multivariate analysis, age >50 (OR: 8.890; 95% CI: 1.662- 47.539), male sex (OR: 4.376; 95% CI: 1.120- 17.100), baseline higher liver stiffness (OR: 7.511; 95% CI: 1.767- 31.934) and higher FIB-4 score (OR: 5.899; 95% CI: 1.292- 26.930) are identified as independent predictors of HCC occurrence in patients after achieving SVR within 6 months after DAAs for HCV infection. (Table 5)

Discussion

The presence of advanced fibrotic or cirrhotic microenvironment is believed to trigger the start and promotion of neoplastic molecular and cellular clones in liver through genetic damage and cellular transformation, ending with HCC (10, 11). To a great extent this believe holds true

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as the risk of HCC occurrence still persists in cirrhotic patients even after eradication of HCV by DAAs (12).

Tolloid like-1 protein is thought to be involved in liver fibrogenesis and in turn hepatocarcinogenesis via activation of profibrogenic and prooncogenic signaling pathways as transforming growth factor-B, insulin-like growth factor and extracellular matrix assembly regulation (13, 14). Moreover, a recent genome-wide association study (GWAS) reported TLL1 gene variants was associated with occurrence HCC after eradication of HCV by Interferon (IFN) based therapy *(15)*

To know the extent of credibility of these data, this study evaluated the relationship between TLL1 and early development of HCC, within 6 months, in Egyptian HCV cirrhotic patients after HCV eradication. Fifty patients with denovo HCC occurrence and another 50 patients without were included. TLL1 rs17047200 genotype was AA in 59%, AT in 25%, TT in 4% and undetermined in 12% of all enrolled patients. There was no significant difference found between the 2 groups regarding the distribution of different TLL1 rs17047200 genotype variants (AA, AT, TT and undetermined). Also, TLL1 rs17047200 genotype variants were not recognized as factors or predictors for early HCC development after SVR with DAAs in univariate and multivariate analyses. Also, there was no relationship between Rs17047200 in TLL1 gene variants and baseline demographic and laboratory findings in HCC group including, age, sex, liver function tests, AFP, MELD score, FIB-4 score, liver stiffness or type of DAAs received. Lastly, there was no significant association found between different TLL1 rs17047200 alleles and morphological features or aggressiveness of HCC as portal vein thrombosis, extrahepatic spread, number and size of HCC nodules. Patients in HCC group showed significantly higher mean age and INR; significantly higher median total bilirubin, MELD score, liver stiffness and FIB-4 score and significantly higher percentage of male gender and diabetes than those in Non HCC group. On multivariate analysis, age >50, male sex, higher FIB-4 score and liver stiffness at baseline were identified as independent predictors of development of HCC in patients achieving SVR within 6 months after DAAs for HCV infection.

To the best of our knowledge, only 2 studies investigated the association between Rs17047200 in TLL1 gene variants and development of HCC after HCV eradication using DAAs (8, 9). In accordance with our results, an Italian retrospective study (9) evaluated the association between Rs17047200 in TLL1 gene variants and risk of HCC development in a large group of HCV cirrhotic patients treated with DAAs. Four hundred and fifty-two cirrhotic patients

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enrolled in this study, 87% of them were Child class A. During 33 months of follow-up from DAA start, de-novo HCC was found in 6.9% of patients. Rs17047200 in TLL1 gene variants was AA in 73%, AT in 26% and TT 1%. AA and AT/TT patients were compared, there were no significant differences according to clinical and laboratory data at baseline. Male sex (P = 0.008), diabetes (P= 0.001) and FIB-4 (P= 0.001) were baseline-independent predictors of HCC development. No significant difference found between patients carrying the different genotype according to the main HCC features, as hepatic focal lesion size, number, AFP and Barcelona clinic liver cancer (BCLC) stage. According to those results, they concluded that Rs17047200 in TLL1 gene variants didn't influence HCC incidence following HCV eradication by DAAs nor HCC features at diagnosis.

On the other hand, our results were not compliant with a Japanese study (8) which included 1029 patients who treated with interferon free regimens and had no history of HCC before treatment and were monitored for one year at least from the end of treatment. This study reported that 19 patients in HCC group developed HCC and 1010 in non-HCC group did not during the observational period with median 104 weeks after they had achieved SVR. HCC group (47.4%) showed significantly higher proportion of rs17047200 AT/TT than the non-HCC group (20.1%) (P = 0.008). Multivariate analysis showed that higher levels of AFP(P = 0.021), FIB-4(P = 0.036) and rs17047200 AT/TT (P = 0.026)were independent risk factors for HCC occurrence Cumulative incidence of HCC was significantly higher in patients with rs17047200 AT/TT than in those with AA (P = 0.006). Clinical characteristics were compared according to the TLL1 genotypes, patients with rs17047200 AT/TT had significantly higher levels of FIB-4 (P = 0.011) and lower platelet counts than those with AA (P = 0.032). Finally the authors concluded that rs17047200 variant was independently associated with HCC occurrence after HCV treatment by oral antiviral drugs and may be implicated in the progression of cirrhosis and thus carcinogenesis.

The explanation of this discrepancy might be due to several factors. Firstly, our study is a prospective study while the Japanese one (8) was retrospective. Secondly, number of our cohort who examined for rs17047200 alleles is smaller. In addition, the variants frequency difference

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between ethnic groups could reduce the effect of rs17047200. Moreover, patients in the Japanese one (8) were followed-up for a long period of time than patients in our study. This was intended from the start of the study as we encountered significant number of cases who developed aggressive HCC within short period after HCV eradication by DAAs and we tried to evaluate any factors which might contribute to this early occurrence. In the end, other risk factors affecting HCC (i.e. diabetes and alcohol) may be involved in HCC development in the Japanese and Egyptian populations. However, one of the strength points in our study is the homogeneity of included population and the prospective design which allow us to follow up the patients as well as the availability of complete patients and HCC data.

Interestingly, our study and both the Italian (9) and the Japanese (8) cohorts found that higher baseline FIB-4 score was independently associated with denovo HCC occurrence after HCV treatment with DAAs. Therefore, the more advanced liver fibrosis and cirrhosis the more risk to develop HCC, despite of genetics.

Limitation:-

There was some limitation in our study first, the Number of our cohort who examined for rs17047200 alleles is smaller with small duration of follow-up. In addition, the variants frequency difference between ethnic groups could reduce the effect of rs17047200. So, further studies on a large group of patients and longer follow up is recommended.

In conclusion, TLL1 genotypes was not related to early HCC development after viral eradication of HCV related cirrhotic patients who treated with DAA. The most agreed on contributing factor for HCC occurrence in this setting is the degree of hepatic fibrosis/ cirrhosis at baseline.

Main points:-

- HCC represents an important public health problem in Egypt. Highly effective DAAs dramatically decrease HCV related liver disease progression to end-stage liver disease and HCC.
- The risk of developing HCC continues to persist in those patients with HCV cirrhosis even after they have achieved SVR.

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- Conflicting results have been published in western and eastern population regarding association between tolloid-like 1 gene (TLL1) variant rs17047200 and HCC development in patients treated and cured with DAAs
- Single Nucleotide polymorphism (SNP) rs17047200 in TLL1 gene was not associated with early HCC development or morphological features and aggressiveness of HCC after viral eradication of HCV related cirrhotic Egyptian patients who treated with DAA.

- **Author contribution**: All authors contributed equally to the work

- **Conflict of interest:** None of the authors have a conflict of interest

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- **Ethics Committee Approval:** - The study was approved by the Research and Ethics Committee of Ain Shams University, Cairo, Egypt in accordance with local research governance requirements and with the Helsinki Declaration of 1975.

- **Informed Consent:** All included patients provided written informed consent

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Table 1: comparison between the 2 groups regarding baseline demographic and laboratory findings

Baseline parameter		Non HCC group (n=50)		HCC group (n=50)		Test value	value-P
		.No	%	.No	%		
Smoking	Non smoker	29	%58.0	31	%62.0	*0.167	0.683
	Smoker	21	%42.0	19	%38.0		
Diabetes	No	34	%68.0	13	%26.0	*17.704	0.000
	Yes	16	%32.0	37	%74.0		
Child class & score	A5	50	%100.0	45	%90.0	*5.263	0.072
	A6	0	%0.0	4	%8.0		
	B7	0	%0.0	1	%2.0		
Treatment received	SOF/DACLA	37	%74.0	37	%74.0	*0.000	1.000
	SOF/DACLA/RIBA	13	%26.0	13	%26.0		
Sex	Female	28	%56.0	13	%26.0	*9.301	0.002
	Male	22	%44.0	37	%74.0		
Age (years)	Mean ± SD	12.70 ± 54.02		6.59 ± 58.98		**2.450-	0.016
AST (IU/ml)	Median (IQR)	(40 – 35) 39		(40 – 37) 39		‡0.705-	0.481
ALT (IU/ml)	Median (IQR)	(66 – 25) 36.5		(37 – 18) 42.5		‡0.910-	0.301
Total bilirubin (mg/dl)	Median (IQR)	(0.9 – 0.5) 0.6		(1.4 – 0.6) 0.8		2.754-	0.006
Albumin (g/dl)	Mean ± SD	0.62 ± 4.00		0.58 ± 3.86		**1.176	0.243
(l/Platelets (10^9	Mean ± SD	44.96 ± 171.1		34.469 ± 170.2		**0.1008-	0.459
Creatin (mg/dl)	Mean ± SD	0.19 ± 0.87		0.19 ± 0.86		**0.155	0.877
AFP (ng/ml)	Median (IQR)	(4.9 – 1.6) 2.66		(5.8 – 2) 3.55		‡0.969-	0.333
(IU/ml) PCR	Median (IQR)	– 390) 4000 (1313305		– 4000) 208938.5 (1313305		‡1.223-	0.221
INR	Mean ± SD	0.15 ± 1.10		0.27 ± 1.21		**2.669-	0.009
FIB4	Median (IQR)	(3.42 - 4.05) 3.66		(3.49 - 4.83) 4.2		‡2.0392--	0.041
MELD	Mean ± SD	1.56 ± 5.48		1.80 ± 6.40		**2.748-	0.007
Stiffness (kpa)	Median (IQR)	(14 – 18) 15		(15 – 26.15) 19.5		‡3.303-	0.0009

***:Chi-square test; **: Independent t-test, ‡: Mann Whitney Test AFP: alpha fetoprotein; ALT: alanine transferase; AST: aspartate transferase; INR: international normalized ratio; MELD: model for end stage liver disease; PCR: polymerase chain reaction**

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Table 2 comparison between HCC and non HCC patients regarding Rs17047200 in TLL1 gene variants identification after treatment:-

		Non-HCC group		HCC group		-P value	.Sig
		.No	%	.No	%		
Rs17047200 in TLL1 gene	Undetermined	**7**	**%14.0**	**5**	**%10.0**	**0.167**	NS
	Homogenous	**27**	**%54.0**	**36**	**%72.0**		
	Heterogenous	**16**	**%32.0**	**9**	**%18.0**		
Call	Undetermined	**7**	**%14.0**	**5**	**%10.0**	**0.300**	NS
	A/A	**25**	**%50.0**	**34**	**%68.0**		
	A/T	**16**	**%32.0**	**9**	**%18.0**		
	T/T	**2**	**%4.0**	**2**	**%4.0**		

Table 3: association between Rs17047200 in TLL1 gene and HCC characteristics at time of HCC diagnosis.

Characteristics of HCC lesions in group A after treatment			Rs17047200 in TLL1 gene			Test value	P value
			Undetermined No=5	Homogenous No= 36	Heterogeneous No= 9		
		Number (%)	(%) Number	(%) Number	(%) Number		
Portal vein thrombosis	Yes	**(%12) 6**	**(%40) 2**	**(%8.3) 3**	**(%11.11) 1**	**4.177**	**0.123**
	No	**(%88) 44**	**(%60) 3**	**(%91.7) 33**	**(%88.89) 8**		
Lesion size	**3**cm≥	**(%78) 39**	**(%80) 4**	**(%80.5) 29**	**(%66.67) 6**	**0.822**	**0.662**
	3cm<	**(%22) 11**	**(%20) 1**	**(%19.5) 7**	**(%33.33) 3**		
Extrahepatic spread	No	**(%94) 47**	**(%80) 4**	**(%97.22) 35**	**(%88.89) 8**	**2.817**	**0.244**
	Yes	**(%6) 3**	**(%20) 1**	**(%2.78) 1**	**(%11.11) 1**		
Number of Lesions	Uninodular	**(%82) 41**	**(%80) 4**	**(%86.11) 31**	**(%66.67) 6**	**1.859**	**0.394**
	Multinodular	**(%18) 9**	**(%20) 1**	**(%13.89) 5**	**(%33.33) 3**		

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Table 4 association between rs17047200 in TLL1 genotypes and baseline clinical and biochemical features of patients in HCC group:-

HCC group		Rs17047200 in TLL1 gene			Test value	value-P
		Undetermined	Homogenous	Heterogenous		
Sex	Female	**(%60.0) 3**	**(%19.4) 7**	**(%33.3) 3**	***4.060**	**0.131**
	Male	**(%40.0) 2**	**(%80.6) 29**	**(%66.7) 6**		
Age	Mean ± SD	**9.69 ± 56.60**	**6.12 ± 60.36**	**4.82 ± 54.78**	***1.211**	**0.149**
DM	No	**(%20.0) 1**	**(%27.8) 10**	**(%22.2) 2**	***0.219**	**0.896**
	Yes	**(%80.0) 4**	**(%72.2) 26**	**(%77.8) 7**		
Child score	A5	**(%80.0) 4**	**(%94.4) 34**	**(%77.8) 7**	***5.076**	**0.280**
	A6	**(%20.0) 1**	**(%2.8) 1**	**(%22.2) 2**		
	B7	**(%0.0) 0**	**(%2.8) 1**	**(%0.0) 0**		
Treatment received	SOF, DACLA	**(%80.0) 4**	**(%75.0) 27**	**(%66.7) 6**	***0.364**	**0.834**
	SOF, DACLA/ RIBA	**(%20.0) 1**	**(%25.0) 9**	**(%33.3) 3**		
AST	Median (IQR)	**(40 – 35) 36**	**(40 – 39) 39**	**(40 – 34) 39**	**‡1.378**	**0.502**
ALT	Median (IQR)	**(33 – 16) 18**	**(38.5 – 20) 28**	**(36 – 17) 23**	**‡0.780**	**0.677**
Total bilirubin	Median (IQR)	**(1.1 – 0.4) 0.7**	**– 0.6) 0.8 (1.35**	**(1.5 – 0.7) 1.3**	**‡1.545**	**0.462**
Albumin	Mean ± SD	**0.59 ± 4.04**	**0.59 ± 3.86**	**0.56 ± 3.76**	****0.376**	**0.689**
PLT	Mean ± SD	**80.95 ± 232.20**	**74.06 ± 225.42**	**96.58 ± 267.22**	****1.011**	**0.372**
Creat	Mean ± SD	**0.19 ± 0.83**	**0.20 ± 0.89**	**0.15 ± 0.78**	****1.214**	**0.306**
AFP	Median (IQR)	**(3.39 – 2.2) 2.2**	**– 1.55) 3.7 (5.8**	**(4.5 – 2) 3.6**	**‡0.208**	**0.901**
INR	Mean ± SD	**0.16 ± 1.12**	**0.30 ± 1.23**	**0.16 ± 1.18**	****0.447**	**0.642**
FIB4	Median (IQR)	**– 3.31) 3.53 (4.13**	**– 3.34) 3.92 (4.03**	**(4.39 – 3.5) 3.61**	**‡1.128**	**0.569**
MELD	Mean ± SD	**1.09 ± 5.58**	**1.90 ± 6.55**	**1.73 ± 6.27**	****0.658**	**0.523**
Stiffness (kpa)	Median	**14.48**	**16.60**	**12.59**	****1.152**	**0.325**

***:Chi-square test **: One Way ANOVA test; ‡: Kruskal Wallis test**

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Table 5: Univariate and Multivariate Logistic regression analysis for predictors of HCC development after DAAs

	Uni-variety				Multi-variety			
	value-P	Odds ratio (OR)	C.I. for OR **%95**		value-P	Odds ratio (OR)	C.I. for OR **%95**	
			Lower	Upper			Lower	Upper
Age >50	**0.001**	**6.517**	**2.211**	**19.215**	**0.011**	**8.890**	**1.662**	**47.539**
Sex	**0.003**	**3.622**	**1.559**	**8.418**	**0.034**	**4.376**	**1.120**	**17.100**
DM	**0.000**	**6.048**	**2.540**	**14.399**	**0.097**	**2.924**	**0.824**	**10.377**
Total bilirubin >1	**0.004**	**5.062**	**1.703**	**10.050**	**0.514**	**1.617**	**0.382**	**6.842**
INR>1.18	**0.000**	**7.333**	**2.652**	**20.282**	**0.102**	**1.032**	**0.647**	**5.324**
MELD>5.72	**0.010**	**2.901**	**1.288**	**6.534**	**0.680**	**0.719**	**0.150**	**3.449**
Liver stiffness	**0.001**	**4.373**	**1.854**	**10.316**	**0.006**	**7.511**	**1.767**	**31.934**
FIB-4	**0.003**	**4.125**	**1.611**	**10.559**	**0.022**	**5.899**	**1.292**	**26.930**