RESEARCH ARTICLE

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ADAM10 Gene Polymorphism and Its Relationship to Hepatocellular Carcinoma in Egyptian HCV Patients Receiving Direct-Acting Antiviral Therapies (DAAs)

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Abstract

Objective: The aim of this study was to investigate the ADAM 10 rs.653765 SNP genetic polymorphism in the hepatocellular carcinoma occurrence (de novo and post DAAs). **Methods:** This study was conducted on 360 participants divided to 4 groups. Group 1: 90 chronic adult patients infected with HCV received DAAs regimens and evolved HCC during the period of follow up. Group 2: Another 90 HCV patients received the same DAAs regimens and did not show HCC manifestations during the same follow up period. Group 3 included 90 de novo HCC patients (did not receive any DAAs). Finally, 90 apparently healthy participants as group 4. Clinical and laboratory data were evaluated, and ADAM 10 genotyping were performed using qPCR. **Results:** The study showed statistically significant between HCC de novo and HCC deterioration on top of DAAs according to three scoring systems (Child Pugh, BCLC and HKLC) with p- value <0.05. Regarding ADAM10 gene polymorphism, the study showed a significant difference between CC versus CT+TT genotypes of HCC groups according to Child Bugh, BCLC and HKLC staging systems. Yet, no significant difference was found when ADAM10 genotypes and allele frequencies were compared between the four different studied groups. No difference in the survival rate between HCC de novo and on the top of DAAs but more aggressive stages with HCC on top of DAAs. **Conclusion:** ADAM10 genotypes did not show any significant association with HCC. Also, no differences in the death rate recorded between the de novo HCC and HCC post DAAs treatment with statistical significant worse staging of HCC post DAAs and were noted. the study showed a significant difference between CC versus CT+TT genotypes of HCC groups according to Child Bugh, BCLC and HKLC staging systems.

Keywords: ADAM10 SNP- Hepatocellular Carcinoma- HCV- Direct Acting Antivirals (DAAs)

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Introduction

Hepatocellular carcinoma (HCC) is the most prevalent type of primary liver cancer in adults and the second most prominent cause of cancer deaths worldwide (Bray et al., 2015) Chronic liver problems such as chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcohol-related liver disease, non-alcoholic fatty liver disease (NAFLD), and cirrhosis are leading triggers of HCC (Huang et al., 2021; Nguyen et al., 2022).

Prevention and control of HCV has been found to be a complex process due to many factors such as the geographic distribution, defining the risk factors associated with the HCV infection and determination of cofactors that affect its progression (Perz and Alter, 2006).

Lately, treatment of HCV with the new direct acting antiviral agents (DAAs) was proved to be effective, safe, and well-tolerated with a sustained virological response rate (SVR) exceeding 95% in many cases providing therapeutic hopes for many patients especially those with higher risk of developing liver failure and HCC (Zhang et al., 2016).

Current treatment regimens for HCV infection include the NS3 protease inhibitor 'simeprevir' (SMV) or the NS5B polymerase inhibitor 'sofosbuvir' (SOF) in combination with peginterferon (PegIFN) and ribavirin

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(RBV). Daclatasvir (DAC) plus PegIFN/RBV has been approved for treatment as well (Gonzalez-Moreno and Payeras-Cifre, 2013; Mahmood et al., 2018).

Better knowledge of the underlying processes of pathophysiology of HCC can be defined by identification of polymorphism of modifier genes such as Murine double minute 2 (MDM2) and other genes that plays a vital role in the p53 pathway (Hosen et al., 2021), several cytokine genes such as Transforming growth factor-β (TGF-β) (Nomair et al., 2021) and Interleukin-10 (Ghanm et al., 2021), and several metalloproteinases such ADAMs.

ADAMs (A-disintigrin and metalloproteinases) is a family of multifunctional membrane bounded proteins involved in cell adhesion and proteolysis (Murphy, 2008). They synchronize various cell surface molecules like growth factors, cytokines/chemokines and adhesin molecules. ADAM10 is a member of the ADAMs family with its characteristic proteolytic shedding to cell surface proteins. It is involved in many aspects of cancer development such as tumour growth, cell migration, and metastasis (Mullooly et al., 2015). The human ADAM10 gene is located on chromosome 15 at position 15q21.3-q23 and it contains 16 exons interrupted by 15 introns (Prinzen et al., 2005). Some researchers have studied the potential role of ADAM10 single nucleotide polymorphisms (SNPs) in progression and regulation of severe sepsis (Cui et al., 2015), tumours like colon cancer (Gavert et al., 2005), and oral squamous cell carcinoma (Jones et al., 2013). ADAM10 is overexpressed in HCC cell lines and significantly associated with the progression of the tumour (Zhang et al., 2014).

These data collectively suggest the potential role of ADAM10 as a tumour marker in cancer detection. However, the exact role of ADAM10 SNPs genotyping in cancer progression generally or HCC development specifically has not been fully investigated. Thus, we aimed in this study to investigate the correlation between the ADAM10 SNPrs653765 as a potential biomarker for HCC susceptibility and deterioration process.

Materials and Methods

This study included 360 participants divided into 4 groups

Group 1: 90 adult patients infected with HCV, received couple of DAAs regimens (Sofosbuvir 400 mg tab once daily and Daclatasvir 60 mg tab once daily) for 12 weeks provided by the outpatients clinic at National Liver Institute, Menoufia University, Egypt. These patients presented with HCC during a follow up period of 36 months.

Group 2: Another 90 HCV patients received the same DAAs regimens as group 1 and did not develop HCC during the same follow up period.

Group 3: included 90 denovo HCC patients (did not receive DAAs) recruited from HCC clinic at National Liver Institute, Menoufia University. Lastly; 90 apparently healthy participants volunteered as controls (Group 4).

The study was approved by the National Liver Institute ethical committee and an informed written consent was obtained from all participants. All of them were enrolled according to following inclusion criteria: positive for anti-HCV antibody, positive HCV RNA (by PCR) higher than1000 IU/ml. Age: not less than 18 years. Antinuclear antibody (ANA) titre less than 1/60.

Exclusion criteria

History of chronic or acute inflammatory disease in the preceding 3 months, malignancies, autoimmune diseases, proof of liver disease decompensation, seropositive for HIV and HBsAg and/or other causes of liver disease. Moderate to severe anaemia (Haemoglobin<10 g/dl), neutropenia (neutrophil count<2000/mm^3), thrombocytopenia (PLT<75000/mm^3) and significant history of neuropsychiatric and cardiovascular diseases.

All participants underwent the following

Full history taking and clinical examination, assessment of the general condition, arterial pulse, blood pressure. Diagnosis of liver cirrhosis and HCC was done by ultrasonography and computed tomography or MRI (magnetic resonance imaging) and relevant laboratory findings.

Laboratory Investigations

blood samples were collected and analysed for blood chemistry, haematology, and HCV RNA. laboratory biochemical investigations were done on (SYNCHRON CX9ALX, Randox, CA, USA). Alpha fetoproteins (AFP) was done on Architect i1000SR, Abbott, USA, and haematology parameters were done using automated Sysmex KX-21 (Sysmex Corporation, Kobe, Japan).

For Molecular analysis

Genomic DNA was extracted from anticoagulated (EDTA) whole blood using a spin column method (Gene jet whole blood genomic DNA purification Mini kit) according to the manufactures protocol (Thermo Fisher Scientific, Lithuania).

For SNP analysis of ADAM10 SNPrs653765 TaqMan allelic discrimination Assay was used to detect variants of a single nucleic acid sequence. The presence of two primer/probe pairs in each reaction allows genotyping of the two possible variants at the SNP site in a target template sequence. The allelic discrimination assay classifies unknown samples as follows:

(1) Homozygotes

samples with only allele 1 or allele 2.

(2) Heterozygotes

samples with both allele 1 and allele 2.

Using genotyping assay (primers and probe) 40 x (Thermo Fisher Scientific, MA,USA), the volumes of reaction master mix for amplification was 20 ul constituted of 0 .5 µl of genotyping assay, 10 µl of genotyping qPCR Master Mix, 3.5 µl of DNase-free water and 6µl of genomic DNA template was added. For the negative control reaction 6ul of DNase-free water was added instead of genomic DNA.

The PCR and genotyping were performed on real time fast 7500 system (Applied Biosystems, Life Technologies

CA, USA), the cycling parameters were set as follows:
Initial denaturation step (holding stage)
95°C for 10 minutes

Cycling stage
35 cycles of denaturation step: 95°C for 15 seconds
Annealing, extension and reading the fluorescent dyes at 60°C for 1 minute.

Statistical analysis of the data
Data were fed to the computer and analysed using IBM SPSS software package version 25. Sample size calculation was done using Epidemiologic Statistics for Public Health (Epi), Online Version (www.OpenEpi.com), and were presented using methods of Kelsey, and Fleiss with a continuity correction. The study included four groups together completing the whole sample size of 360 participants of the study.

Qualitative data were presented in the form of numbers (N) and percentages (%). It was analysed by chi-square (χ^2) test. However, if an expected value of any cell in the table was less than 5, Fisher Exact test was used (if the table was 4 cells). Mann Whitney U test was used to compare quantitative data that was not normally distributed. However, Kruskal Wallis test was used instead in more than 2 groups. Log rank test was used to assess difference of survival distribution between the two HCC studied groups. Significance levels were set at 5% level.

The genotype frequencies of the studied genes were compared with predictable values calculated from Hardy-Weinberg equilibrium ($p^2+2pq+q^2 = 1$; where p is the frequency of the wildtype allele and q is the frequency of the variant allele).

Sample size calculation
Epi-info website was used to calculate sample size for unmatched case control study.

Hardy Weinberg equilibrium genotypes distribution in control group.

Genotyping	Observed Frequency	Expected Frequency	Chi-square Test	P value
CC	5	8.1		
CT	44	37.8	2.421	0.119
TT	41	44.1		

Results

Comparison of HCC denovo and HCC on top of treatment, HCV and control groups regarding their medical history, clinical presentation, and laboratory investigations. The mean age + SD were 52.9± 4.56, 48.97± 0.76, 47.96± 1.12 and 48.71± 1.1) in the four groups consequently. In addition, there was a statistically highly significant difference between mentioned study groups regarding history of hypertension where (46.7%) of HCC denovo group suffered from hypertension and that (43.3%) of them were diabetics, p value= (0.05, 0.005) consequently. In addition (95.6%) of study participants in the HCC on top of treatment study group experienced clinical history of decompensation. There was a statistically significant difference between HCC denovo and HCC on top of treatment study groups regarding presence of clinical history of decompensation (p-value<0.05). It also showed a highly statistically significant difference between the four study groups regarding their ALT, AST, AFP, Total bilirubin, ALB, and INR (P value =0.000) Table 1.

When staging was carried out for the study groups utilizing the three scoring systems; Child Pugh, Barcelona Clinic Liver Cancer (BCLC) and Hong Kong Liver Cancer (HKLC), the results in Table 2 showed that (90%) of HCC de novo patients were in child score (A 5,6) and (40%) of them were in HKLC stage (IIIa), where (56.7%) were in

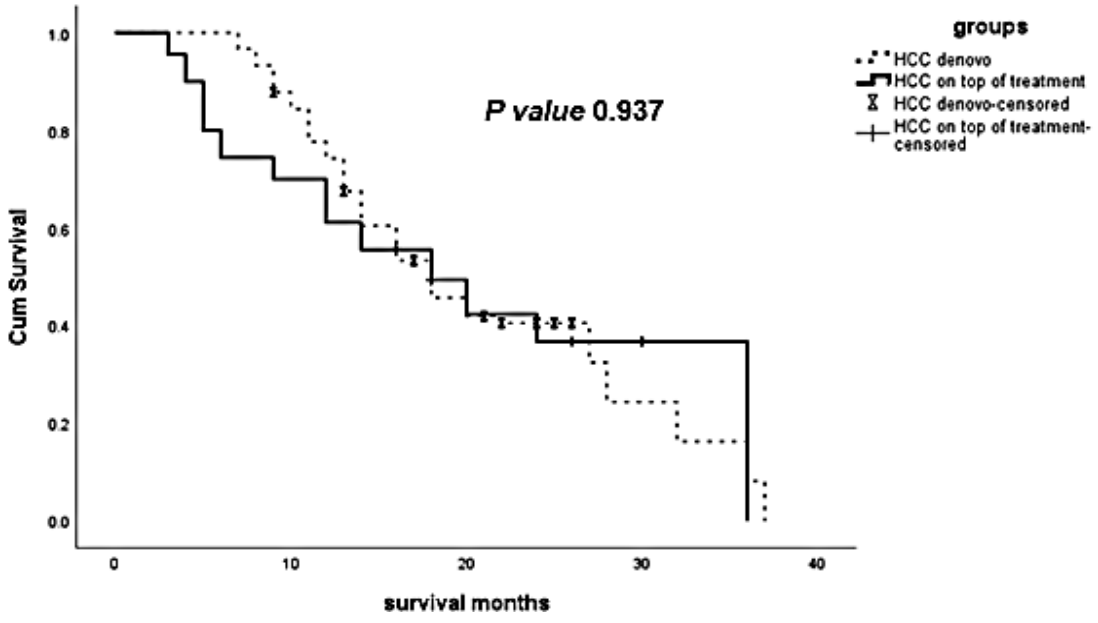

Figure 1. Survival Distribution in HCC de novo and HCC on Top of Treatment Groups.

Table 1. Comparison of Study Groups Regarding Their Sociodemographic, Medical History and Clinical Presentation

Group		HCC denovo N=90	HCC on top of treatment N=90	HCV N=90	Control N=90	Test of significance	P-value
Age (years)						0.814*	0.487
Mean ± SD		52.9± 4.56	48.97± 0.76	47.96± 1.12	48.71± 1.1		
ALT(U/L)							
Mean ± SD		58.80± 36.80	28.52± 13.80	35.53± 19.45	16.29± 3.57	157.10*	<0.01
AST(U/L)							
Mean ±SD		59 ± 36.17	35.43± 18.05	34.50±14.5	16.37±3.78	171.203*	<0.01
AFP(ng/dL)							
Median		399	750.5	25.25	3.25	582.44*	<0.01
Range		8731	68201	117.9	8.5		
Total Bilirubin(mg/dL)							
Median		1	1	0.6	0	187.333*	<0.01
Range		2	2	1.5	1		
ALB(gm/dL)							
Mean ± SD		3.80± 0.60	3.79± 0.609	4.33± 0.36	4.55± 0.68	112.03*	<0.01
INR							.
Median		1	1	1	1	29.93*	<0.01
Range		1	1	1.03	0.5		
Diabetes	Positive	39 (43.3%)	37 (41.1%)	30 (33.3%)	20 (22.2%)	10.57**	0.05
	Negative	51 (56.6%)	53 (58.9%)	60 (66.7%)	70 (77.8%)		
Hypertension	Positive	42 (46.7%)	25 (27.8%)	27 (30%)	18 (20%)	12.69**	0.005
	Negative	48 (53.3%)	65 (72.2%)	63 (70%)	72 (80%)		
Clinical	Positive	78 (86.7%)	86 (95.6%)	1 (1.1%)	NA	3.36**	0.03
Decompensation	Negative	12 (13.3%)	4 (4.4%)	89 (98.9%)			
Focal lesion	Positive	27 (30%)	17 (18.9%)	NA	NA	3.00**	0.08
	Negative	63 (70%)	73 (81.1%)				
Cirrhosis	Positive	87 (96.7%)	75 (83.3%)	32 (85.6%)	NA	91.89**	0.08
	Negative	3 (3.3%)	15 (16.7%)	58 (64.4%)			
Lymph node	Positive	12 (13.3%)	20 (22.2%)	6 (6.6%)	NA	1.29**	0.11
	Negative	78 (86.7%)	70 (77.8%)	84 (93.3%)			

*, Kruskal Wallis test; **, Chi square test

(stage B) in BCLC scoring system. Comparable findings were found in the HCC group on top of treatment where the higher percent of patients scored A 5,6 in the Child Pugh system (75.6%) and 52.2 % were in HKLC stage (IIIa), where (36.7%) were in (stage B) in BCLC scoring system. As for the genotyping distribution among the three different scoring systems, there was a highly significant difference between CC versus CT+TT genotypes of both HCC study group regarding Child Pugh, BCLC and HKLC staging systems with p value 0.007, 0.001, 0.004 respectively. Statistically significant worse staging of HCC post DAAs were noted.

When the genotypes and alleles frequency were compared in table 3 for the four study groups there were no significant difference between the groups (p value >0.05) regarding their distribution.

No statistically significant difference was found in the survival rate when both HCC de novo and HCC on top of treatment groups were compared (p value > 0.05). The mean survival time was (20.877, 20.516) months in HCC denovo and HCC on top of treatment study groups consequently. These data were illustrated in Figure 1.

Discussion

Direct acting antivirals are the promising and agreed therapy for HCV with less side effects and more than 90% success rate. Unforeseen controversy between higher rate of HCC after DAAs was reported by Muzica and co-workers (2020). This needs more research to discover the true effect of DAAs on HCC. We aimed to study ADAM10 gene polymorphism in Egyptian HCV patients receiving direct-acting antiviral therapies.

ADAMs play a crucial role in cellular behaviour in different tumours including their ability of multiplication, invasion, progression, distant metastasis, and cellular life span (Yuan et al., 2013; Minond, 2020). ADAM10 polymorphism rs653765 C > T is associated with the progression of hepatocellular carcinoma (Shiu et al., 2018)

The current study showed that 95.6% of HCC

Table 2. Comparison of the Study Groups and Genotypes According to the Hepatocellular Carcinoma and Cirrhosis Scoring Systems (Child Pugh, BCLC and HKLC).

Variables	HCC de novo	HCC on top of treatment	Likeli hood ratio (LR)	P value	Genotyping		Likelihood Ratio (LR)	P value
					CC	CT+TT		
	N (%)	N (%)			N (%)	N (%)		
Child score			6.713*	0.03 S				
A(5)	81 (90)	68 (75.6)			12(48)	102 (65.8)	14.014	0.007 HS
A(6)					9 (36)	26 (16.8)		
B (7)	6 (6.7)	13 (14.4)			0 (0.0)	8 (5.2)		
B (9)					4 (16)	7 (4.5)		
C (10)	3 (3.3)	9 (10.0)			0 (0.0)	12 (7.7)		
BCLC staging			12.768	0.01 S				
0	6 (6.7)	0 (0.0)			6 (24)	28 (18.1)	10.62	0.001 HS
A	15 (16.7)	13 (14.4)						
B	51 (56.7)	47(52.2)			19 (76)	79 (51)		
C	15 (16.7)	21 (23.3)			0 (0.0)	36 (23.2)		
D	3 (3.3)	9 (10.0)			0 (0.0)	12 (7.7)		
HKLC staging			13.453	0.03 S			19.073	0.004 HS
I	18 (20.0)	9 (10.0)			6 (24)	21 (13.5)		
II a	0 (0.0)	4 (4.4)			0 (0.0)	4 (2.6)		
III a	36 (40.0)	33 (36.7)			12 (48)	57 (36.8)		
III b	18 (20.0)	14 (15.6)			7(28)	25 (16.1)		
IV a	12 (13.3)	16 (17.8)			0 (0.0)	28 (18.1)		
IV b	3 (3.3)	9 (10.0)			0 (0.0)	12 (7.7)		
V b	3 (3.3)	5 (5.6)			0 (0.0)	8 (5.2)		
Total	90 (100)	90 (100)			25 (100)	155 (100)		

*, Chi square test

patients on top of treatment with DAAs experienced clinical history of decompensation and 90% of them suffered from splenomegaly and there was a statistically significant difference between HCC denovo and HCC on top of treatment study groups regarding the presence of clinical history of decompensation and splenomegaly (p-value<0.05). In addition, there was a statistically highly significant difference regarding history of hypertension. There was numerical difference between studied HCC groups regarding portal vein invasion, lymph nodes, multifocal lesion, cirrhosis, and metastasis. In agreement with these results ElFayoumie et al., (2020) assured the aggressiveness of HCC post DAAs regarding portal infiltration, lymph nodes involvement, multifocal lesion and metastasis.

Meringeret and co-workers (2019) reported that HCC on top of DAAs prevailed in older age group and in cirrhotic patients, while in the present study, the high significant differences between the four study groups were seen regarding their ALT, AST, and AFP (P value =0.000). On the other hand, ElFayoumie et al. (2020) did not report such difference between their studied HCC groups in ALT and AST levels.

Our study showed a statistically significant difference between HCC denovo and HCC on top of DAAs when they were classified according to Child Pugh, BCLC and HKLC staging systems, more advanced stages in HCC on top of treatment declaring more aggressiveness. Our observations tuned with Yang et al., (2020) who found that 7% of HCC were in C stage of BCLC and 2% were in stage C of Child Pugh classification.

On the other hand, Zanetto et al., (2018) found it

Table 3. Comparison of Study Groups Regarding Genotypes and Allele Frequency

Genotyping	Groups				P value
	HCC de novo N (%)	HCC on top of tretment N (%)	HCV N (%)	Control N (%)	
CC	12 (13.3)	13 (14.4)	15 (16.7)	5 (5.6)	
CT	33 (36.7)	47 (52.2)	42 (46.7)	44 (48.9)"	
TT Alleles	45 (50.0)	30 (33.3)	33 (36.7)	41 (45.6)	0.07 NS
C allele	57 (31.7)	73 (40.6)	72 (40)	54 (30)	
T allele	123 (68.3)	107 (59.4)	108 (60)	126 (70)	0.06 NS

hard to acknowledge the aggressiveness of HCC on top of DAAs due to multi-factors such as small sample sizes. Moreover, Shiha et al., (2020) demonstrated that hepatocellular carcinoma on top of DAAs treatment had less aggressiveness than denovo hepatocellular carcinoma.

In the present study a highly statistically significant difference was found between CC genotype versus CT +TT genotypes of HCC study groups regarding the Child Pugh, BCLC and HKLC scoring systems. Similar to our results, Shiu et al. (2018) described correlation between risky genotypes of ADAM10 SNP rs653765 and advanced stages of Child Pugh classification, lymph nodes and distant metastasis.

Different research groups provided theories about the associations between ADAM10 serum level and HCC via the proteolytic effect of ADAM10 supporting its role in cancer invasion and metastasis (Uehara et al., 2018).

This study showed no significant difference between study groups regarding their ADAM10 genotypes and their distribution among the two HCC studied groups. Shiu et al. (2018) also showed no statistically significant difference between HCC group and control groups regarding their genotypes.

Within the limitation of the current study, we can conclude that ADAM10 genotypes did not show any significant association with HCC and there is no difference in the death rate recorded between the de novo HCC and HCC post DAAs treatment but there was a highly statistically significant difference between CC genotype versus CT +TT genotypes of HCC study groups regarding the Child Pugh, BCLC and HKLC scoring systems and significant difference between HCC on top of treatment and HCC de novo with more advanced stages in HCC on top of treatment declaring more aggressiveness. Further studies with larger sample size may elucidate this correlation.

Author Contribution Statement

Ghanem S. and El Gedawy G. performed the laboratory work and computations. Yehia S performed sample size calculation, the statistical analysis of the study data and verified the analytical methods. Bedair H and Awad S verified the analytical methods. Abdel-Razek W. and Elhelbawy M collected the research samples and helped in writing the manuscript. EL-Sabaawy D. helped in writing and submitting the manuscript. ElFert A. developed the theory and supervised the findings of this work and verified the analytical methods.

All authors discussed the results and contributed to the final manuscript.

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Approval

If it was approved by the institutional review board of National Liver Institute, Clinical biochemistry and molecular diagnostics department.

Ethical Declaration

It was approved by the research ethics committee of the National Liver institute, Menoufia University, Egypt (NLI IRB 00003413), approval no. 0110/2020.

Data Availability

patients' laboratory results, clinical record and radiological images were collected from National Liver Institute Hospital and Clinics, Menoufia University, Egypt.

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