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Research Article

Significance of DEPDC5 and MICA Variants in Hepatocellular Carcinoma risk related Hepatitis C Virus patients in Egypt

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Abstract

Objectives: Hepatitis C virus (HCV) infection is one of the major causes of hepatocellular carcinoma (HCC) in Egypt. Recently, genetic polymorphisms of DEPDC5 and MICA have been reported to correlate with the progression of HCC in hepatitis C patients. The aim of this study was to determine the relationship between DEPDC5 (rs1012068) T/G, MICA (rs2596542) C/T and the risk of HCC development in patients with HCV infection.

Methods: One hundred HCV infected patients suffering from HCC and one hundred healthy controls were enrolled in the current study. Single nucleotide polymorphisms (SNPs) have been studied for DEPDC5 and MICA using real-time PCR.

Results: Out of the two genes polymorphisms analyzed, the DEPDC5 and MICA variants were significantly related to the development of HCC ($p<0.0001$). Only the DEPDC5 variants showed a high ($p<0.0001$) significant difference in patients with cirrhosis. Moreover, the DEPDC5 variants were significantly correlated with low platelets count ($p<0.045$).

Conclusion: DEPDC5 (rs1012068) and MICA (rs2596542) could be a valuable indicator in diagnosing the progression of liver disease to HCC risk related Hepatitis C Virus patients in Egypt.

Keywords: Egypt, DEPDC5, hepatocellular carcinoma, hepatitis C virus, MICA

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Worldwide, hepatocellular carcinoma (HCC) is the most recognized threatening tumor of the liver. HCC represents about 70% to 85% of primary liver cancers.[1] It is the third major reason of cancer death in men and the fourth in women with more than 600.000 deaths annually.[2] In Egypt, HCC is the second most common cancer in men and the sixth most common cancer in women.[3] The incidence of HCC has doubled in the past ten years in Egypt.[2] There is a direct correlation between the occurrence of HCC and hepatitis C virus (HCV) or hepatitis B virus (HBV) infection, indicating that these two viral infections are the major risk factors of HCC all over the world.[4] Co-infection of these two viral infections is related to a higher risk for HCC progress than mono-infection.[5]

Egypt has one of the highest prevalence rates of HCV in the world with about 10% chronic HCV infection among people aged 15 to 59 years.[6] Natural clearance of HCV is reported to take place in more than one-third of the acute infections whereas two-thirds of patients will progress to chronic HCV infection.[7, 8] Around 15% of chronically infected HCV patients

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develop to liver cirrhosis and finally, 5% of these liver cirrhosis patients might develop to HCC through five years.[9–11]

Previous reports identified two specific genetic factors to HCV-related HCC in Japanese patients, the Dishevelled, Egl-10 and Pleckstrin Domain-Containing 5 (DEPDC5) locus and the MHC class I polypeptide-related sequence A (MICA) locus.[12, 13] DEPDC5 gene is located on the long arm of chromosome 22, 22q12.3 and it encodes a cytoplasmic protein which has been lately shown to have an important function in focal epilepsy, a neurological disorder.[14] The function of DEPDC5 has not been identified until now; nevertheless, DEPDC5 protein has been established to block the effect of mammalian target of rapamycin (mTOR), a multi-functional protein participated in many cellular processes including inflammation, growth of the cell and tumor formation including hepatocarcinogenesis.[15–17] Although the role of this gene is still unidentified,[11, 18] it is notable that DEPDC1, which contains a DEP domain analogous to DEPDC5, has been assumed to have an effect on bladder cancer.[19, 20]

MICA SNP rs2596542 is localized on chromosome 6p21. This SNP has been localized inside the class I major histocompatibility complex (MHC) region and is around 4.8 kb upstream of MHC class I polypeptide-related sequence A gene.[12] MICA is a membrane protein that is up-regulated in various cancer cells and also stimulated in response to various cellular stresses such as infection, hypoxia, and heat shock.[21] It is an essential part of the innate immune response, where MICA can be linked to the natural killer group 2 member D (NKG2D) receptor and then activate NK cells and $CD8^+$ cells.[22, 23]

The aim of the current study was to investigate the association of the DEPDC5 (rs1012068) and MICA (rs2596542) with HCC risk in HCV-related HCC cases in Egypt.

Subjects and Methods

Subjects

One hundred HCC cases were recruited from National Cancer Institute, Cairo University and one hundred healthy controls were recruited from National Liver Institute, Menoufia University (controls were age and gender-matched with cases) in the period from February 2017 to November 2017. Written consent was signed from all recruited subjects and the institutional ethical committee of the National Liver Institute approved the study.

Sample Collection

Ten ml of venous blood was withdrawn from all cases and controls under a complete aseptic condition in EDTA vacutainer tubes. All samples were centrifuged at 3000 rpm for 10 min, plasma and buffy coat were separated, aliquoted and stored at −80°C till testing.

Methodology

Plasma samples were used for the serological testing of HCV Ab, HBsAg, and AFP using ADVIA Centaur CP (Switzerland) according to the manufacturer instructions. Assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), and Albumin (ALB) was measured using Beckman CX4 chemistry analyzer (NY; USA). Hemoglobin (HB) and platelets were measured using Sysmex XT-1800i (Japan). Also, plasma was used to perform in-house RT HCV PCR for the detection of HCV.[24] Compensated cirrhosis was assessed in patients by Fibro-scan™ (EchoSens™, Paris, France) as >12.5 kPa.

Genomic DNA was isolated from the buffy coat of cases and healthy control samples using a DNA isolation kit (QiAamp DNA mini kit, Qiagen, Hilden, Germany). Amplification of genomic DNA samples was used to detect the genetic polymorphisms of DEPDC5 (rs1012068) and MICA (rs2596542).[12, 13] Allelic discrimination probes of each gene were labeled with either FAM or VIC as fluorescent dyes using real-time PCR System (Applied Biosystems: Foster City, CA, USA). The PCR reaction was carried out using a TaqMan universal master mix (Applied Biosystems: Foster City, CA, USA) at a primer/probe concentration of 1X. The reaction was performed in a 96-well format in a total reaction volume of 25 µl using 20 ng of genomic DNA. The reaction was heated for 2 min at 50 °C, then 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1.5 min. The fluorescence intensity of each well in the TaqMan assay plate was read in each cycle and the amplification plot was constructed by the real-time machine. Allelic discrimination was evaluated automatically by the software (SDS Software v2.3, USA).

Statistical Analysis

Statistical analysis was performed using SPSS 20 (Chicago, Inc, Illinose). Quantitative data were shown as mean and standard deviation (SD) or expressed as frequency and percent. Chi-square test and Fisher exact test were used to measure the association between qualitative variables as appropriate. Mann Whitney and independent sample t-tests were done to measure the association between two quantitative variables as appropriate. The Kruskal-Wallis and ANOVA tests were done to measure the association between more than two quantitative variables as appropriate.

Results

The demographic, serological and biochemical data of the recruited subjects are presented in table 1. No significant difference in age and gender was found between cases and controls in ($p=0.365$ and 0.626 respectively). All HCC cases were positive for HCV-Ab and HCV-RNA by RT-PCR, while healthy controls were all negative. Both cases and controls were negative for HBsAg. The levels of AFP, ALT, AST, and

TBIL were significantly higher in HCC cases than in controls. On the other hand, ALB, the platelet count and HB concentration were significantly lower in HCC cases than controls.

The genotyping results of DEPDC5 (TT as the wild genotype), (TG as heterozygous genotype), and (GG as mutant genotype) are shown in the table 2. The distribution of genotypes detected in HCC cases was as follows 23 cases (23.0%) with TT genotype, 49 cases (49.0%) with the TG genotype and 28 cases (28.0%) with the GG genotype; respectively. On the other hand, the distribution of genotypes detected in healthy controls was as follows 71 cases (71.0%) with TT genotype, 24 cases (24.0%) with the TG genotype and 5 cases (5.0%) with the GG genotype; respectively. The difference between cases and controls in DEPDC5 genotypes was highly significant (p<0.0001). The genotyping results of MICA (CC as the wild genotype), (CT as heterozygous genotype), and (TT as mutant genotype) are shown in the table 2. The distribution of genotypes detected in HCC cases was as follows 13 cases (13.0%) with the CC genotype, 58 cases (58.0%) with the CT genotype and 29 cases (29.0%) with the TT genotype; respectively. Controls showed a significantly different genotype distribution (p<0.0001) as follows 40 cases (40.0%) with the CC genotype, 49 cases (49.0%) with the CT genotype and 11 (11.0%) with the TT genotype; respectively.

Table 1. Clinicopathological characteristics of the studied groups

	HCC n=100	Control n=100	p
Age	57.63±8.338	54.23±8.729	0.365
Gender, (%)			
Male	73 (73.0)	76 (76.0)	0.626
Female	27 (27.0)	24 (24.0)	
HCV Ab, (%)			
Positive	100 (100.0)	0 (0.0)	<0.0001*
Negative	0 (0.0)	100 (100.0)	
HCV PCR, (%)			
Positive	100 (100.0)	0 (0.0)	<0.0001*
Negative	0 (0.0)	100 (100.0)	
HBsAg, (%)			
Negative	100 (100.0)	100 (100.0)	
AFP (ng/ml)	618.4±472.1	6.7±6.0	<0.0001*
ALT (IU/L)	63.5±33.3	13.0±6.0	<0.0001*
AST (IU/L)	67.2±50.6	15.3±4.9	<0.0001*
TBIL (mg/dL)	3.0±2.6	0.6±0.3	<0.0001*
ALB (g/dL)	3.0±0.5	4.1±0.4	<0.0001*
HB (g/dL)	10.8±1.2	13.4±0.8	<0.0001*
Platelets (10^3/µL)	116.6±38.4	227.0±37.4	<0.001*

Data are presented in terms of Mean±SD in terms of numbers of patients (%). *P-value <0.05 was considered significant.

No significant correlation (p=0.065) were found between gender and DEPDC5 genetic variants (TT, TG, and GG), which represented 13 (56.5%), 36 (73.5%), 24 (85.7%) in male patients; 10 (43.5%), 13 (26.5%), and 4 (14.3%) in female patients, respectively. Interestingly, a higher percentage was detected with male patients than females. No significant correlation (p=0.378) was found between age and DEPDC5 genetic variants, regarding the different polymorphisms in the age category ≤50 or >50 years, but it is obvious that higher percentage was recorded with aged patients. Highly significant association (p<0.0001) was found in liver cirrhosis of the various genetic variants of DEPDC5, where 4 (17.4%) cirrhotic cases were found in TT polymorphism, 16 (32.7%) in TG polymorphism, and 16 (57.1%) in GG polymorphism, abnormal polymorphism showed a high percentage compared to normal one as shown in table 3.

Table 2. Comparison of genotype and allele distributions of DEPDC5 and MICA, between the studied groups

	HCC n=100	Control n=100	p
DEPDC5, (%)			
Genotypes			
TT	23 (23.0)	71 (71.0)	
TG	49 (49.0)	24 (24.0)	<0.0001*
GG	28 (28.0)	5 (5.0)	
MICA, (%)			
Genotypes			
CC	13 (13.0)	40 (40.0)	
CT	58 (58.0)	49 (49.0)	<0.0001*
TT	29 (29.0)	11 (11.0)	

Data are presented in terms of numbers of patients (%). *P-value <0.05 was considered significant.

Table 3. The gender, age, and liver cirrhosis comparison among the DEPDC5 genotypes in the HCC group

	DEPDC5			p
	TT n=23	TG n=49	GG n=28	
Gender, (%)				
Male	13 (56.5)	36 (73.5)	24 (85.7)	0.065
Female	10 (43.5)	13 (26.5)	4 (14.3)	
Age, (%)				
≤50 years	3 (13.0)	13 (26.5)	5 (17.9)	0.378
>50 years	20 (87.0)	36 (73.5)	23 (82.1)	
Liver cirrhosis, (%)				
36/100	4 (17.4)	16 (32.7)	16 (57.1)	<0.0001*

Data are presented in terms of numbers of patients (%). *P-value <0.05 was considered significant.

A significant correlation (p=0.045) was also found between DEPDC5 SNP variants and platelets count but none of the other biochemical parameters showed such a significant correlation (AFP, ALT, AST, TBIL, ALB, and HB) (Table 4).

No significant correlation (p=0.827) was found between gender and MICA genetic variants (CC, CT, and TT) which represented 10 (76.9%), 41 (70.7%), 22 (75.9%) in male patients; 3 (23.1%), 17 (29.3%), and 7 (24.1%) in female patients, respectively. Interestingly, a higher percentage was demonstrated with male patients than females. No significant correlation (p=0.286) was found between age and MICA genetic variants regarding the different polymorphisms in age category ≤50 or >50 years, but it is obvious that higher percentage was recorded with aged patients. No significant correlation (p=0.484) was found in liver cirrhosis among the different MICA genetic variants as shown in table 5.

There is no significant correlation between MICA SNP variants and biochemical parameters investigated among the patient's group as shown in table 6.

Discussion

It is well recognized that HCV infection is affected by host factors such as male gender, older age, obesity and host genetics.[25] As a result, there is rising attention in the identification of host genetic genotypes that might have a role in the patient's response to infection and disease severity. Lately, many genome-wide association studies (GWAS) were conducted on chronic HCV-infected patients suffering fibrosis, cirrhosis with or without HCC.[13, 26, 27] These studies were carried out to predict the clinical outcome of the infection and provide a guide for controlling the development of HCC in patients infected with HCV. These studies identified possible prognostic genetic factors including polymorphisms in DEPDC5 and MICA genes that were related to HCV-infected patients with HCC.

In the current study, the mean age of HCC patients was (57.63±8.338) years. These findings are in accordance with the study done by Abdel-Wahab et al. (2007) who noted that the mean age of HCC patients was (54.26±9.2) years.[28] Regarding the gender of HCC patients, the present study showed that HCC risk was more common among male than female, out of 100 HCC cases 73 (73%) were male and 27 (27%) were female. These finding agreed with a study done by do-Carmo et al. (2012) who found a remarkable occurrence of HCC in male patients.[29] Investigating the laboratory parameters among HCC patients compared to control groups showed statistically significant differences between the two groups for AFP, ALT, AST, TBIL, ALB, HB and platelets count, which come in line with Ripoll et al. (2009) and Hanafy et al. (2016).[30, 31]

In the present study, we investigated the association between DEPDC5 rs1012068 T/G and the risk of HCC in Egyptian patients with HCV infection. Our study demonstrates

Table 4. The laboratory findings among the DEPDC5 genotypes in the HCC group

	DEPDC5			p
	TT n=23	TG n=49	GG n=28	
AFP (ng/ml)	474.8±484.2	527.9±481.7	631.5±499.5	0.494
ALT (IU/L)	40.1±33.6	57.6±39.7	60.6±30.9	0.161
AST (IU/L)	46.1±34.0	66.6±45.0	71.5±67.7	0.253
TBIL (mg/dL)	3.3±3.7	2.4±1.8	2.0±1.4	0.223
ALB (g/dL)	3.2±0.50	3.0±0.4	2.8±0.4	0.057
HB (g/dL)	11.2±1.07	10.7±1.3	10.5±0.9	0.169
Platelets (10^3/µL)	132.5±34.5	117.2±38.4	102.0±37.7	0.045*

Data are presented in terms of Mean±SD. * P-value <0.05 was considered significant.

Table 5. The gender, age and Liver cirrhosis comparison among the MICA genotypes in the HCC group

	MICA			p
	CC n=13	CT n=58	TT n=29	
Gender, (%)				
Male	10 (76.9)	41 (70.7)	22 (75.9)	0.827
Female	3 (23.1)	17 (29.3)	7 (24.1)	
Age, (%)				
≤50 years	2 (15.4)	10 (17.2)	9 (31.0)	0.286
>50 years	11 (84.6)	48 (82.8)	20 (69.0)	
Liver cirrhosis				
36/100	5 (38.5)	17 (29.3)	14 (48.3)	0.484

Data are presented in terms of numbers of patients (%).

Table 6. The laboratory findings among the MICA genotypes in the HCC group

	MICA			p
	CC n=13	CT n=58	TT n=29	
AFP (ng/ml)	545.8±496.6	549±492.1	535.7±485.6	0.993
ALT (IU/L)	44.7±29.8	55.4±38.5	56.7±36.3	0.643
AST (IU/L)	53.7±26.2	67.7±63.4	59.2±29.7	0.659
TBIL (mg/dL)	2.47±1.7	2.5±1.9	2.6±3.5	0.968
ALB (g/dL)	3.0±0.2	3.0±0.5	3.0±0.4	0.877
HB (g/dL)	11.0±1.1	10.8±1.3	10.7±1.0	0.825
Platelets (10^3/µL)	127.0±30.0	115.5±40.6	113.6±38.6	0.622

Data are presented in terms of Mean±SD.

that the frequency of the G allele of rs1012068 was more frequent in the HCC patients than in the healthy control group suggesting the strong association between the G allele and HCC progression which is almost similar to the Japanese study by Miki et al. (2011) who conducted a GWAS between 212 patients with HCV-related HCC and 765 chronic hepatitis C (CHC) without HCC where they identified the intronic SNP rs1012068 located in DEPDC5 is significantly associated with susceptibility to HCC.[13] The same group recorded that the mRNA levels of DEPDC5 were higher in tumor tissues compared to non-tumor tissues.[13] Our results come in agreement with Motomura et al. (2012) who stated the DEPDC5 minor allele was more susceptible to HCC development in Asian subjects.[26] Also, Al-Anazi and his colleagues (2014) had demonstrated that DEPDC5 SNPs rs1012068 was associated with chronic HCV infection and with end-stage liver disease progression in Saudi patients.[11]

However, a study was done by Hai et al. (2017) who did not confirm the relationship between the DEPDC5 rs1012068 and the development of HCC in Japanese patients with chronic hepatitis C.[32] Another study was done by Burza et al., (2016) in Europeans patients with cirrhosis caused by HCV infection and tracked them up for HCC progress and did not find any relationship between DEPDC5 rs1012068 and HCC occurrence. The different results of these studies clarified that DEPDC5 rs1012068 might be population-dependent.[33]

To increase the power of our analysis, we investigated the association between the genotypes of the DEPDC5 rs1012068 and liver cirrhosis; we observed a highly significant association ($p<0.0001$) of DEPDC5 rs1012068 and cirrhosis in this group of patients with cirrhosis. Our results suggest that DEPDC5 rs1012068 increases the susceptibility to cirrhosis in HCC patients with HCV infection. Our data are the same as a study carried out by Burza et al. (2016) who reported an association between severe fibrosis (cirrhosis) and DEPDC5 rs1012068.[33] Moreover, a study from Saudi Arabia on HCV-positive patients reported that patients carrying the heterozygous genotype for DEPDC5 SNP rs1012068 were more likely to be cirrhotic or with HCC.[11]

In addition, investigating the effect of DEPDC5 rs1012068 on disease outcomes by linking the different genotypes of DEPDC5 rs1012068 T/G with AFP as a tumor marker and many liver function parameters, there was no significant correlation between these genotypes and clinical parameters such as AFP, liver enzymes (ALT, AST), TBIL, and HB. However, there was a significant difference ($p=0.045$) only between DEPDC5 variants and platelets count. Our results suggest that DEPDC5 rs1012068 GG genotype had a higher frequency in patients with low platelets count.

The MICA gene is expressed in most epithelial cancer cells, including HCC, lung cancer, breast cancer, and prostatic cancer.[22, 34–36] So, its relationship with malignancies has become a research focus of many researchers. MICA is a ligand for NKG2D, exerts its anti-tumor effect by activating natural killer cells and CD8+ T cells.[22] In our study, we found a significant difference ($p<0.0001$) in MICA polymorphisms distribution between HCC patients and healthy controls. Therefore, there is an association between MICA gene polymorphism and HCC in Egyptian patients infected with HCV. Our study is similar to those done by Mohamed et al. (2017) who demonstrated that the T allele contributed to increased risk of HCC development in HCV infected patients.[37] Our results are also equivalent to Hoshida et al. (2012) who suggested that the frequency of the T risk allele was higher in HCC patients against the control.[38] Another study by Li et al., 2016 has shown that HCV patients with the allele T of rs2596542 were more susceptible to HCC than the allele C and the TT genotype of MICA rs2596542 polymorphism.[36] In agreement with our results, a study carried out by Hai et al. (2017) who determined the risk allele of rs2596542 in MICA is likely to play a role in HCC development.[32] The Previous study found a strong association between MICA rs2596542 and HCV-induced HCC.[12] Moreover, the expression of MICA mRNA was reduced in HCC patients.[39]

Investigating the effect of the SNP rs2596542 on the disease progression showed that the three SNP rs2596542 genotypes showed no significant association with liver cirrhosis in the studied population. This finding is in accordance with Mohamed et al., 2017 who found that rs2596542 C/T genetic variation is not a significant marker to HCC development in Egyptian patients with liver cirrhosis.[37] However, our result is in disagreement with results from earlier research by Hai et al. (2017) who suggested that the risk allele of MICA may associate with HCC in patients with fibrosis which might indicate ethnicity dependent effect of this SNP on the progression of HCV infection.[32] Also, we found no association between MICA rs2596542 alleles and laboratory parameters such as AFP, liver enzymes (ALT, AST), total bilirubin, ALB, HB and platelets, and this comes in agreement with Motomura et al. (2012) and Mohamed et al. (2017).[26, 37]

In the current study a higher percentage of all studied genes, SNP variant was detected in male aged patients than female younger ones. Several studies found an obvious association between age, sex, and HCC or HCV infection.[28, 29, 40] An increased carrier rate of hepatitis among males than females can be explained by the higher expression

of androgen receptors in HCC rather than the expression of estrogen receptors and the possible genetic predisposition.[41] The significant association between DEPDC5, MICA and HCC related-HCV infection indicates an association between these genes and age/gender of HCC subject. Therefore, high frequencies of the studied genes were observed in male aged HCC patients.

Conclusion

In conclusion, DEPDC5 and MICA are associated with the development of HCC in Egyptian patients with HCV infection.

Disclosures

Ethics Committee Approval: The study was approved by the Local Ethics Committee (NLI-001.09.2017/1).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.A-H, H.M.I; Design – M.A-H, H.M.I, I.A.E; Supervision – M.A-H, H.M.I, I.A.E, H.S.G; Materials – E.L, M.M.E, H.S.G; Data collection &/or processing – H.M.I, E.L, M.M.E, H.S.G; Analysis and/or interpretation – H.M.I, E.L, M.M.E, M.A-H; Literature search – E.L, M.M.E, H.S.G; Writing – H.M.I, E.L, M.M.E; Critical review – M.A-H, H.M.I, I.A.E.

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