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Original Article

Clinical significance of SNP (rs2596542) in histocompatibility complex class I-related gene A promoter region among hepatitis C virus related hepatocellular carcinoma cases

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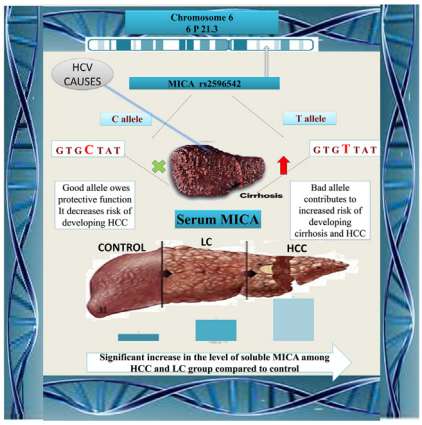
GRAPHICAL ABSTRACT



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ABSTRACT

The major histocompatibility complex class I-related gene A (MICA) is an antigen induced by stress and performs an integral role in immune responses as an anti-infectious and antitumor agent. This work was designed to investigate whether (SNP) rs2596542C/T in MICA promoter region is predictive of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) or not. Forty-seven healthy controls and 94 HCV-infected patients, subdivided into 47 LC and 47 HCC subjects were enrolled in this study. SNP association was studied using real time PCR and soluble serum MICA concentration was measured using ELISA. Results showed that heterozygous genotype rs2596542CT was significantly ($P = 0.022$) distributed between HCC and LC related CHC patients. The sMICA was significantly higher ($P = 0.0001$) among HCC and LC. No significant association ($P = 0.56$) between rs2596542CT genotypes and sMICA levels was observed. Studying SNP rs2596542C/T association with HCC and LC susceptibility revealed that statistical

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significant differences ($P = 0.013$, $P = 0.027$) were only observed between SNP rs2596542C/T and each of HCC and LC, respectively, versus healthy controls, indicating that the rs2596542C/T genetic variation is not a significant contributor to HCC development in LC patients. Moreover, the T allele was considered a risk factor for HCC and LC vulnerability in HCV patients (OR = 1.93 and 2.1, respectively), while the C allele contributes to decreasing HCC risk. Therefore, SNP (rs2596542C/T) in MICA promoter region and sMICA levels might be potential useful markers in the assessment of liver disease progression to LC and HCC.

Introduction

Chronic infection with HCV is a predisposing factor to cirrhosis and chronic liver disease (CLD), which has been described as the most important precursor to Hepatocellular carcinoma (HCC) [1]. HCC is the fifth abundant type of tumors worldwide and the third lethal cancer, causing 600,000 deaths each year [2]. The Human Leukocyte Antigen (HLA) system owns specific function in adaptive immune response against viral and tumor antigens [3]. HLA region, located on chromosome 6p21.3 includes nearly 4 mega base segment developed through a repetitive doubling of genes and conversion [4]. The MICA and MICB proteins are MHC class I homologs that do not have a role in antigen presentation [5] but both along with UL 16-binding proteins, serve as ligands for immunostimulatory C type lectin like receptor NKG2D, expressed in most NK cells and CD8 positive T cells and gamma delta T cells [6,7]. The human major histocompatibility complex class I chain A related gene (MICA) has been recognized and described by Fodil in 1996 on the short arm of chromosome 6 within the MHC-I region [8]. The highly polymorphic MICA protein is expressed due to stress as that caused by heat shock or particular bacterial and/or viral infections and was restricted to endothelial keratinocytes [7,9]. MICA protein also stimulates the immune function in the mucosal tissues; it binds to NKG2D, and then initiates a series of signals. NKG2D mediated tumor rejection is based on 2 mechanisms [10]: (I) The expressed NKG2D ligand on cancer cells strongly stimulates the NK cell effector functions, even surpassing inhibitory signals by MHC class I molecules. (II) Promotes lysis of tumor cells by CD8-T cells through the promotion of T cell receptor signaling. The shedding of soluble MICA is associated with a simultaneous decrease in NKG2DL expression of cell surface resulting in reduced immunostimulatory signals for cytotoxic lymphocytes [11]. Furthermore, soluble MICA is connected with systemic down regulation of NKG2D on CD8T surface along with gamma and delta T cells, to prevent the antitumor action of such cells [12]. The Genome Wide Association Study (GWAS) found that a formerly identified locus in the flanking region of MICA at codon 50, which is located upstream of MICA gene by 4.7 kb on chromosome 6p21 (rs2596542) to be strongly associated with HCV induced HCC. In spite of the fact that the molecular mechanism by which this single nucleotide polymorphism (SNP) is correlated with HCC progression remains unclear, MICA SNPs were suggested to affect antitumor immunity [9]. The significance of SNP (rs2596542) lies through its absolute linkage in the MICA promoter region and which can change the binding of stress inducible transcription factors [13]. Tong et al. [14] hypothesized that SNP rs2596542 may potentially alter the expression of MICA or initiate pathways related to tumor progression. Given high levels of endemic CHC infection in Egypt and that SNP rs2596542 is a potentially important factor, a better understanding of correlations of the SNP rs2596542 with LC and HCC among Egyptian patients infected with HCV genotype 4 is required. The aim of the study was to investigate the prevalence of the SNP rs2596542 C/T among some HCV patients with LC and HCC compared to healthy controls and to test for the significance of soluble MICA serum levels with regards to the prevalence of HCV-related LC and HCC. Also, the impacts of different variables, as host characteristics (age, gender, etc.) on the MICA SNP rs2596542 frequencies and hepatic disease progression were studied.

Patients and methods

The experimental protocols were conducted after understanding and obtaining written consent forms from the study subjects (patients and healthy controls). The National Hepatology and Tropical Medicine Research Institute ethical review board approved the study protocol. Ninety-four Egyptian HCV infected patients were enrolled in this study at the National Hepatology and Tropical Medicine Research Institute. Based on clinical, biochemical and serological parameters, patients were divided into two subgroups, liver cirrhosis related chronic hepatitis C (LC, n = 47) and hepatocellular carcinoma related chronic hepatitis C (HCC; n = 47). All subjects were confirmed positive for HCV-Ab and negative for anti-HBV and anti-HIV. Forty-seven healthy Egyptian blood donors were recruited as the healthy control group (n = 47). All control individuals were also confirmed negative for HBV and HCV antibodies by routine serology and none of these individuals had any history of alcohol intake or drug use.

Biochemical analysis

Fasting venous blood samples (~7 mL) were collected by trained laboratory technicians. Assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin, albumin kits were obtained from (Diamond Diagnostics, Cairo, Egypt), Creatinine using Creatinine Assay Kit (Abcam, Cambridge, UK), glucose concentrations using Glucose Assay Kit (Abcam, Cambridge, UK) and alpha fetoprotein (AFP) on subjects' sera using a Beckman CX4 chemistry analyzer (NY; USA). Viral status (HbsAg and anti-HCV) were measured using Abbott; Axyam (USA). Complete blood picture was carried out on subjects' plasma. Quantification of soluble serum MICA (sMICA) levels by ELISA in patients and healthy controls was performed using ELISA kits for human sMICA (Thermo Fisher, Boston, MA, USA). The lowest detection limit for sMICA proteins was 20 pg/mL. For genotyping of SNP (rs2596542) in the MICA promoter region, genomic DNA was isolated from peripheral blood mononuclear cells using DNA isolation kit (QiAamp DNA mini kit: Qiagen, Hilden, Germany). DNA samples from patients and control subjects were genotyped for SNP rs2596542 C/T using TaqMan ViiA 7 Real Time PCR System (Applied Biosystems: Foster City, CA, USA). One of the allelic probes was labeled using FAM dye and the other with the fluorescent VIC dye. The PCR reaction was carried out using a TaqMan universal master mix (Applied Biosystems: Foster City, CA, USA) at a probe concentration of 20X. 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 plates were heated for

2 min at 50 °C and for 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1.5 min. The fluorescence intensity of each well in TaqMan assay plate was read.

Statistical analysis

Quantitative data were statistically represented in terms of minimum, maximum, mean, standard deviation (SD) and median. Comparison between two groups was done using independent sample *t*-test or Mann-Whitney test. Comparison between >2 groups was conducted using an ANOVA test or Kruskal-Wallis test (non-parametric ANOVA). Qualitative data were statistically represented in terms of numbers and percentages. Comparison between different groups was done using Chi-Square Test and Relative Risk and Odds ratio. Receiver operating characteristic curve (ROC) was applied to generate the cutoff value, Area under the curve (AUC), sensitivity and specificity. A probability value (*P* value) less than or equal to (0.05) was considered significant. All statistical analyses were performed using statistical software SPSS (Statistical Package for Social Science). Graphs were done using SPSS statistical program version (16.0) and Microsoft Excel program version 2010.

Results

Demographic analysis of the study cohort revealed statistical significant difference between the groups by gender. The dominance of males were observed in the HCC group while the females were greater in the control group. Results of MICA SNP (rs2596542) C/T genotype distribution among control and patient (HCC and LC) groups showed that there was a significant difference among the three groups regarding SNP rs2596542C/T distribution ($P = 0.022$) as presented in Table 1. Allelic distribution showed that the C allele was significantly higher among the control group while the T allele was significantly higher among the HCC group ($P = 0.025$). Assessment of soluble MICA level revealed that there was a significant increase in the level of soluble MICA in HCC and LC groups compared to healthy controls as shown in Table 1. No significant association was observed between rs2596542CT genotypes and sMICA levels ($P = 0.566$) as presented in Table 2. A relationship between the examined SNP rs2596542 C/T with HCC and LC susceptibility was observed as shown in Table 3. The association of rs2596542CT and rs2596542TT genotypes taking rs2596542CC as a reference among HCC group versus control group ($P = 0.005$, $P = 0.013$), LC group versus control group ($P = 0.050$, $P = 0.030$) and HCC group versus the LC group ($P = 0.350$, $P = 0.660$) was studied. There was no significant difference in the comparisons of HCC versus LC regarding the genotype and allele frequencies while there were statistical significant differences among HCC versus control and LC versus control. Nevertheless, allele C rs2596542C was observed more frequently in control group in comparison to HCC and LC patients where, (OR = 2.1, (95%CI = 1.17–3.78), $P = 0.013$) and (OR = 1.93, 95% CI = (1.07–3.46), $P = 0.027$), respectively, as shown in Table 3. Studying SNP rs2596542 C/T with biochemical parameters among the patients group showed no observed significant association between the SNP rs2596542CT and some clinical parameters as liver enzymes (ALT, AST) total bilirubin, AFP, ALP, WBCs, Platelets, tumor size, serum creatinine, RBCs, BMI, HB and other parameters. However, there was an association between SNP variants and FBS and platelets as $P = 0.041$ and $P = 0.037$, respectively (Table 4). To elucidate the association between the advance of clinical status and the level of clinical obsessive markers in patients' group, the levels of these pathological markers was measured and was correlated to MICA genotype frequencies.Results showed significant difference only when comparing the levels of AST and AFP with the SNPrs2596542 CC versus TT/CT genotypes ($P = 0.013$ and $P = 0.031$, respectively) as shown in Table 5. Receiver operating characteristic curve (ROC) was carried out to illustrate the sensitivity and specificity of sMICA detection levels for discrimination between HCC or LC patients and healthy volunteers and to identify the area under the curve (AUC) Fig. 1. The cutoff value was set at 209 pg/mL. Out of 97 CHC patients a total of seventy-nine patients (40 HCC and 39 LC) had sMICA levels above the cutoff value versus 3 only from the 47 subjects in control group. AUC under the ROC curve = 0.809, sensitivity = 91.5% and specificity = 83%.

Table 2
Studying soluble serum MICA levels with SNP rs2596542 C/T among the two patient groups (LC and HCC).

	Serum MICA (ng/dL) (Median (Min., Max.))	*P value*
MICA SNP rs2596542		
CC	252 (99–1230)	0.566
CT	246 (85–1365)	
TT	194 (89–1000)	
Allele		
C	252 (85–1365)	0.396
T	230 (85–1365)	
Dominant		
CC	252 (99–1230)	0.703
TT and CT	243 (85–1365)	
Recessive		
CC and CT	252 (85–1365)	0.289
TT	194 (89–1000)	

Data is presented in terms of Median, Minimum and Maximum using non-parametric test Kruskal-Wallis. A *P-value* < 0.05 was considered significant.

Discussion

MHC class I polypeptide related chain A (MICA) molecule belongs to the non-classical class I family and its expression is

Table 1
MICA SNP (rs2596542) CT genotypes, distribution among patients (HCC + LC) and control group and assessment of soluble MICA levels.

MICA SNP (rs2596542)		Control	LC	HCC	*P value*
Genotype[1]	CC No (%)	19 (40.4%)	9 (19.1%)	6 (12.8%)	0.022
	CT No (%)	23 (48.9%)	28 (59.6%)	32 (68.1%)	
	TT No (%)	5 (10.6%)	10 (21.3%)	9 (19.1%)	
Allele[1]	C (%)	(64.9%)	(48.9%)	(46.8%)	0.025
	T (%)	(35.1%)	(51.1%)	(53.2%)	
Serum MICA (ng/dL) (Median (Min., Max.))[2]		110 (85–520)[a]	246 (90–1000)[a]	342 (99–1365)[a]	0.0001

SNP: single nucleotide polymorphism, (LC) Liver cirrhosis and (HCC) hepatocellular carcinoma. A *P-value* < 0.05 was considered significant.
[1] Data is presented in terms of numbers, percentages using Chi square-test (χ^2).
[2] Data is presented in terms of Median, Minimum, and Maximum using non-parametric test Kruskal-Wallis.
[a] The groups, which have the same letters are not significantly different from each other using non-parametric test Mann-Whitney.

Table 3
Association of SNP rs2596542 C/T with HCC and LC.

MICA SNP 2596542	HCC vs. LC		HCC vs. control		LC vs. control		HCC vs. control + LC	
Genotype	OR (95% CI)	*P value*	OR (95% CI)	*P value*	OR (95% CI)	P value	OR (95% CI)	*P value*
CC	Ref.		Ref.		Ref.		Ref.	
CT	1.71 (0.54–5.42)	0.35	4.4 (1.52–12.75)	0.005	2.57 (0.98–6.75)	0.05	2.93 (1.09–7.85)	0.028
TT	1.35 (0.34–5.32)	0.66	5.7 (1.37–23.76)	0.013	4.2 (1.11–16.0)	0.03	2.8 (0.84–9.38)	0.089
Allele								
C	Ref.		Ref.		Ref.		Ref.	
T	1.09 (0.61–1.93)	0.77	2.1 (1.17–3.78)	0.013	1.93 (1.07–3.46)	0.027	1.5 (0.91–2.47)	0.109

Data is presented in terms of numbers and percentages using Chi square-test (χ^2) and Odds ratio with 95% confidence interval (CI). A *P-value* < 0.05 was considered significant. Odds ratios (OR) were calculated for the bad T allele by considering the good C allele as a reference.

Table 4
Studying SNP rs2596542 C/T with biochemical parameters among patients group.

MICA SNP rs2596542	CC (n = 15)	CT (n = 60)	TT (n = 19)	*P value*
Age years (Mean ± S.D.)[1]	59.2 ± 7.42	58.78 ± 8.39	56.37 ± 4.92	0.443
BMI kg (Mean ± S.D.)[1]	26.31 ± 2.71	26.45 ± 2.45	26.49 ± 1.74	0.972
Hb (g/dL) (Mean ± S.D.)[1]	11.75 ± 1.55	10.84 ± 1.79	11.29 ± 2.05	0.184
Albumin (g/dL) (Mean ± S.D.)[1]	2.59 ± 0.53	2.53 ± 0.65	2.57 ± 0.41	0.926
INR (Mean ± S.D.)[1]	1.46 ± 0.33	1.47 ± 0.32	1.44 ± 0.33	0.910
RBCs (cells/mm^3) (Mean ± S.D.)[1]	3.9 ± 0.68	3.63 ± 0.67	3.69 ± 0.57	0.360
FBS (mg/dL) (Median (Min.-Max.))[2]	145 (96–239)[a]	215.5 (95–535)[a]	207 (85–535)[a]	0.041
Platelets (10^3/μL) (Median (Min.-Max.))	91 (49–282)[a]	109 (38–255)[a]	78 (38–156)[a]	0.037
S. Creatinine (mg/dL) (Median (Min.-Max.))[2]	1.2 (0.6–5.8)	1.3 (0.4–6.9)	1.2 (0.6–6.5)	0.542
ALT (IU/mL) (Median (Min.-Max.))[2]	47 (20–153)	46 (2.9–160)	46 (17–122)	0.942
AST (IU/mL) (Median (Min.-Max.))[2]	66 (30–151)	76 (16–394)	72 (24–172)	0.486
AST/ALT Ratio (Median (Min.-Max.))[2]	1.23 (0.91–2.2)	1.39 (0.75–24.83)	1.29 (0.81–3.76)	0.525
Billirubin (mg/dL) (Median (Min.-Max.))[2]	2.16 (0.6–12.6)	2.35 (0.5–99)	2.2 (1–16)	0.943
WBCs (cells/mm^3) (Median (Min.-Max.))[2]	6.7 (2.8–18.7)	7.065 (2.7–98)	5.6 (2.7–15.6)	0.488
ALP (U/L) (Median (Min.-Max.))[2]	147 (61–800)	128 (61–522)	146 (72–235)	0.678
Size (cm)(Median (Min.-Max.))[2]	3.95 (0–7)	3.25 (0–8.5)	4.5 (0–7)	0.482
AFP (10–500 ng/dL) (Median (Min.-Max.))[2]	33 (5.6–1198)	33 (2.5–30,000)	40 (2.5–13,670)	0.416

A *P-value* < 0.05 was considered significant.
[1] Data is presented in terms of Mean ± S.D. using ANOVA test.
[2] Data is presented in terms of Median, Minimum and Maximum using non-parametric test Kruskal-Wallis.
[a] The groups which have the same letters in the same row are not significantly different from each other using non-parametric test Mann-Whitney.

Table 5
Levels of some pathological markers associated with MICA genotypic frequencies in LC and HCC groups.

	AFP (ng/dL) Median (Min.-Max.)	ALT (IU/mL) Median (Min.-Max.)	AST (IU/mL) Median (Min.-Max.)	AST/ALT ratio Median (Min.-Max.)
SNP rs2596542 C/T				
CC	7.6 (2.9–1198)	31 (20–153)	40 (24–151)	1.1086 (0.86–2.2)
TT and CT	20 (2.5–30,000)	40 (2.9–160)	56 (16–394)	1.25 (0.62–24.83)
P value	0.031	0.102	0.013	0.110

Quantitative data were statistically presented in terms of minimum, maximum and median using Mann-Whitney Test. A *P-value* < 0.05 was considered significant.

induced by several stress factors including viral infections [15]. MICA is a membrane protein that acts as a ligand for NKG2D to initiate anti-tumor effects through NK and CD8 + T cells [9]. MICA is released into the serum via cleavage at the transmembrane domain by matrix metalloproteinases [11,16] and represses the anti-tumor effect of NK and CD8+ by hindering their activity [17]. Increased soluble MICA levels have been reported to be linked with several types of tumors, including HCC [18–20]. The single nucleotide polymorphism SNP at restriction site (rs2596542C/T) has an absolute linkage within the MICA promoter region and may alter the binding of stress inducible transcription factors. Tong et al. [14] hypothesized that the SNP rs2596542 could affect the expression of MICA or initiate pathways related with tumor development. Despite the fact that the molecular mechanism by which SNPs in MICA region are associated with HCC development remains unknown, however, they were suggested to affect antitumor immunity [9]. The GWAS found that a previously unidentified locus in the 50 flanking region of MICA, which is found 4.7 kb upstream of the MICA gene on chromosome 6p21 (rs2596542) to be strongly associated with HCV related HCC. Consequent analyses using chronic HCV individuals demonstrated that this SNP is not associated with CHC susceptibility but rather is essentially associated with the progression from CHC to HCC. In this study, the investigation of the associations between SNP (rs2596542C/T) with the risk of HCC occurrence in HCV Egyptian patients was examined.

In the studied cohort, hepatocellular carcinoma and liver cirrhosis were more common among males than females, out of 92 cases 62 were males (65.9%) and 32 were females (34%). This male predominance can be attributed to up regulation of the androgen pathways in male patients, which accelerates liver carcinogenesis while estrogen protects hepatocytes from malignant transformation in females [21]. The mean BMI in the patient group was 26.44 while in the control group was 23, so liver diseases are associated with an increase in BMI. This comes in agreement with Ratziu et al. [22], who reported that the predominance of disease progression among patients with cirrhosis and a history of

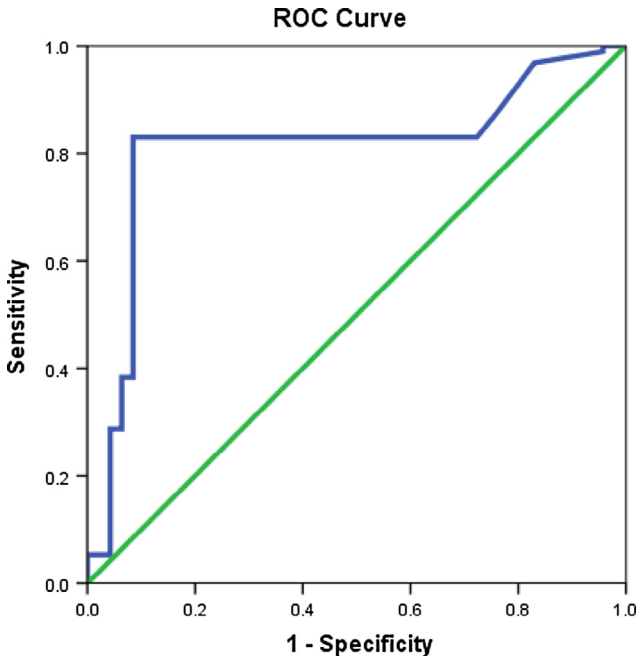

Fig. 1. Roc curve for s MICA serum levels among patients with HCV related HCC and LC. Receiver operating characteristic curve (ROC). The cutoff value was set 209 pg/mL. AUC under the ROC bend = 0.809, sensitivity = 91.5%, and specificity = 83%.

overweight was much higher than among those with cirrhosis and lean body weight. Concerning the laboratory investigations among the patients and control groups (HCC and LC vs. control), there were statistically significant differences between groups in FBG, PLTs, creatinine, albumin, ALT, AST, bilirubin, INR, AFP, haemoglobin, and INR. Where, patients' group had AST levels with a mean 79.2 IU/mL, ALT levels with a mean 52.5 IU/mL and AFP with a mean 1359.7 ng/mL, which were significantly higher than control 33 IU/mL, 32 IU /mL, and 5.8 ng/mL, respectively. Hb level was significantly lower in the patients' group than in the control group (11 ± 1.8 g/dL and 11.8 ± 1.5, respectively). Bilirubin level in the patients' group was significantly higher with a mean level (6) mg/dL when compared to control. Also, the FBG levels were significantly higher (216.3 mg/dL) in comparison to control group which come in line with Hanafy et al. [23], who also reported the increase of these parameters according to liver disease progression. On the other hand, the albumin was significantly lower (2.5 g/dL) in comparison with the control group as found by Ripoll et al. [24] and justified by Spinella et al. [25] who reported that advanced cirrhosis is characterized by diminished albumin concentration as well as impaired albumin functions due to definite structural changes and oxidative damage. The difference in MICA genotype distribution between HCC, LC patients and the healthy controls reached statistically significant level, as shown in Table 1 where (P value = 0. 022) and this comes into agreement with Al-Qahtani et al. [26] and Lange et al. [27]. Thus, there is an association between MICA gene polymorphism and chronic Liver diseases (cirrhosis and hepatocellular carcinoma) in Egyptian patients infected with HCV genotype 4. Assessment of the serum soluble MICA level in HCC, LC, and healthy controls showed that the level of serum MICA increased among progression of liver disease as shown in Table 1. This agrees with previous study reporting that HCV infected people with higher membrane bound MICA level may bring about more immune reaction. The membrane bound mMICA serves as a ligand for NKG2D to stimulate the immune system against viral infected cells by NK and CD8+ cells [9]. The mMICA is then shed by metalloproteinases that are frequently over expressed in cancer tissues and convert mMICA to sMICA, which promotes the tumor formation through the inhibitory effect of sMICA on NK cells. This brought about increased sMICA levels in the sera of HCV patients [28]. Also, soluble MICA is connected with a systemic down regulation of NKG2D expression on the surface of CD8 T cells and gamma, delta T cells, thereby further inhibiting the antitumor effect of such cells [12]. Serum MICA levels are fundamentally higher in sera of patients with different malignancies than in other patients, who in turn reveal higher levels than healthy individuals [29]. In Table 2, the distribution of sMICA levels across MICA variants in patients and controls was examined, notably SNP rs2596542 C/T variants were not significantly associated with sMICA levels, (P = 0.566). Although sMICA levels didn't reach statistically significant levels with the SNP rs2596542 C/T variants, it was noticed that the risk genotype TT was accompanied with lower levels of sMICA. Thus, additional studies are warranted using larger sample sizes to confirm the current findings. Kumar et al. [13] reported that SNP rs2596542 C/T was significantly correlated with sMICA levels, and the risk genotype TT was associated with low levels of sMICA. The association between the risk T allele of rs2596542 with lower sMICA levels in individuals with HCV induced HCC was previously reported [13,30–34]. According to the NCBI map database, the incidence of SNP rs2596542 was recognized in different ethnic population: European (C = 0.726 and T = 0.274), Chinese Han (C = 0.733 and T = 0.267) and Japanese (C = 0.665 and T = 0.335) [26]. While in this study the allele frequency among the control group was (C = 0.649 and T = 0.351) Table 1, which is fairly analogous to that observed among the Japanese population. The frequencies of rs2596542 CC (40.4%), CT (48.9%), and TT (10.6%) in this study came in agreement with Al-Qahtani et al. [26] who reported that, in Saudian population the genotype distribution of MICA rs2596542 CC (32.2%), CT (47.4%), and TT (20.4%). However, in Chinese, the genotype distribution of MICA rs2596542 CC (52.4%), CT (41.9%), and TT (5.7%) [33]. Obviously, there were ethnicity-related variables in the recurrence of rs2596542 polymorphisms. In this study, Egyptians had the heterogeneous CT genotype at SNP rs2596542 more frequent than the protective CC genotype. SNP rs2596542 C/T association with HCC and LC susceptibility was studied, comparing HCC group individually with LC and control groups as well as with a non HCC group (LC + control) in Table 3, revealed that there was no significant difference in the genotype and allele frequencies when comparing HCC versus LC while there was statistically significant difference in the comparisons of HCC versus control and LC versus control, suggesting that rs2596542 C/T genetic variation is not a significant contributor to HCC development in patients with liver cirrhosis i.e. the SNP rs2596542 is not related to progression of HCC from liver cirrhosis. However, rs2596542C allele was observed more frequently in the control group relative to HCC and LC patients indicating that it contributes to decreased risk of HCC, while rs2596542T allele was a risk factor of HCC and LC susceptibility in chronic HCV carriers where, (OR = 2.1, 95% CI = (1.17–3.78), P = 0.013) and (OR = 1.93, 95% CI(1.07–3.46), P = 0.027), respectively, as illustrated in Table 3 and this agrees with Al-Qahtani et al. [26] and Jiang et al. [35]. The T risk allele was more frequent in the patients group (0.521) compared to that of control group (0.351). Also Hoshida et al. [32] reported that the frequency of T risk allele was greater in patients (4.00) compared to that of control (0.331). In the studied cohort, the finding that the T allele of rs2596542 conferred a higher risk for HCC than the C allele, and that it might also be a susceptibility factor for HCC matches with Li et al. [36] who reported that the T allele of rs2596542 had a higher risk for HCC than the C allele (OR = 1.57, 95% CI = 1.07–2.31) and also the TT genotype of MICA rs2596542 polymorphism could raise the risk onset of HCC as it was correlated with the occurrence of the disease. Collectively, the higher

frequency of rs2596542 CC genotype in healthy controls compared to LC and HCC groups suggests a protective role of CC genotype against the progressionofHCV-related livercarcinoma. On the contrary, Aguilar-Olivos et al. [2], Lange et al. [27], and Chen et al. [33] found that the minor T allele of rs2596542 appeared to have a protecting effect on HCC progression, representing an opposite finding as compared to the current study and the results by Kumar et al. [13], which may be attributed to ethnicity-related variations as previously mentioned. Additionally, by examining the influence of the SNP rs2596542 on the disease outcomes by correlating the three SNP rs2596542 C/T genotypes with several liver function parameters and cancer markers as shown in Table 4, it was found that there was no significant association between this SNP variants and clinical parameters such as liver enzymes (ALT, AST), total bilirubin, AFP, ALP, WBCs, Platelets, tumor size, serum creatinine, RBCs, BMI, and HB and this comes in agreement with Motomura et al. [31]. However, there was statistical significant difference only among FBS and platelets ($P = 0.041$ and $P = 0.037$, respectively). SNP rs2596542 TT genotype had higher fasting glucose levels and thrombocytopenia. Also, no significant correlation was found between levels of serum MICA (pg/mL) and tumor size as ($r = 0.1$ and $P = 0.5$), which is in line with Holdenrieder et al. [29], who reported no association between sMICA levels and tumor size ($P = 0.456$), whereas Li et al. [37] reported that sMICA level was correlated with tumor size. ROC curves illustrated the sensitivity and specificity of sMICA levels to discriminate between patients with HCC or LC and healthy volunteers, not for distinguishing between LC and HCC. It was recognized that the serum MICA detected with ELISA had a sensitivity of 83% and specificity of 91.5% at 209 pg/mL cutoff. This cutoff was comparable to Xu et al. [38] who used a cut off = 200 pg/mL. According to these results, sMICA levels possess a good predictive ability as AUC = 0.809.

Conclusions

This study gives thorough data in regards to the clinical status and MICA SNP rs2596542C/T genotype and sMICA levels. It revealed that possession of MICA genotype variants (TT/CT) led to an increased risk of chronic liver disease progression in this cohort. Thus, the T allele contributed to increased risk of HCC development in HCV infected patients and light was shed on the role of MICA genotype as a potential prognostic marker for liver disease progression. Also sMICA levels were significantly higher in the sera of HCC patients than in LC patients, which in turn revealled significantly higher levels than healthy subjects. Therefore SNP rs2596542 C/T genotype and sMICA levels could be potential biomarkers for liver disease progression.

Study limitation

Additional studies are warranted using larger sample size to investigate MICA gene polymorphism and the consequent functional significance in case of HCV infection. Moreover: further longitudinal studies are needed to better confirm the current findings. Studies will progress to illustrate whether the estimation of sMICA level has a prognostic pertinence in threatening maladies.

Conflict of interest

The authors have declared no conflict of interest.

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