Original Article

Assessment of Matrix Metalloproteinase-14 Gene Polymorphisms (+7096) in Egyptian Patients with Hepatocellular Carcinoma

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ABSTRACT

Background:
Hepatocellular carcinoma (HCC) ranks as the first and the second malignancy in men and women in Egypt. Many inflammatory markers are included in HCC pathogenesis. Matrix metalloproteinase-14 (MMP-14) is one of them and it is implicated in regulating HCC tumor progression and prognosis. This study was done basically to assess the frequency of alleles of rs2236307 single nucleotide polymorphism (SNPs) of the MMP-14 (+7096) gene in patients with HCC and to determine if there is an association between its alleles polymorphism and the occurrence of HCC in Egyptian patients.

Materials and Methods:
A case-control study was done in the Tropical Medicine Department at Zagazig University Hospitals and the Medical Biochemistry Department, Faculty of Medicine, Zagazig University. The study included 540 subjects, classified into three groups HCC, cirrhotic, and controls (180 in each group). MMP-14 gene polymorphism analysis in the promoter of the MMP-14 gene rs2236307 was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results:
The TC genotype was significantly higher among patients with HCC when compared with the control group (77.8% versus 16.7%, respectively). The TC genotype was higher among patients with HCC compared with the cirrhotic group (77.8% versus 38.9%, respectively). Metastatic lesions and portal vein thrombosis were significantly higher among the TC group compared with the CC and the TT groups in the HCC group.

Conclusion:
The polymorphisms of MMP-14 rs2236307 increase the risk of HCC. Both the TC and the CC genotypes showed HCC risk association but the the CC genotype appeared with lower potential.

Keywords: Hepatocellular carcinoma, MMP-14 (+7096) rs2236307, TC genotype, CC genotype

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INTRODUCTION

Hepatocellular carcinoma (HCC) is guilty of relatively 841 000 current cases and 782 000 deaths each year globally exhibiting a very pinched prognosis for HCC. The 5-year survival rate is nearly 3% in patients with metastatic HCC in comparison with 31% in patients with localized disease (1).

The pathogenesis of HCC is through multiple and various risk factors with complex interactions between them. These factors include chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), cirrhosis, carcinogen exposure such as aflatoxin B1 (AFB1), excessive alcohol drinking, and a variety of genetic factors (2).

Egypt's unique nature of the liver disease, HCV rather than HBV was linked to the development of HCC, which ranks as the first and the second malignancy in men and women respectively, with a remarkable increase in the proportion of HCC amongst patients with chronic liver disease from 4.0% to 7.2% over a decade (3).

The first distinguished metalloproteinase in the MMP family was MMP-14, which is naturally anchored to the cell membrane in an activated form. MMP-14, also known as the membrane-type MMP (MT1-MMP), has been shown to correlate with a variety of physiological functions and tumor-related behaviors such as migration, invasion, metastasis, basement membrane remodeling, and angiogenesis (4).

The elucidation of various MMPs, including MMP-14, has been implicated in regulating HCC tumor progression and prognosis. Also, MMP-14 is over-expressed in highly invasive HCC (5). Also, HCC cell migration and invasion are inhibited by Targeting MMP-14 (6). Epidemiological studies suggest that genetic factors, including SNPs, are important in mediating an individual's susceptibility to many types of cancer. When a single nucleotide in the shared sequence of a gene differs between the paired chromosomes in an individual, SNPs occur. SNPs in the promoter and coding exon regions of MT1-MMP can alter their binding with transcription factors and gene transcription. Different SNPs, therefore, have different impacts on the expression of genes and their protein and exhibit different risks to develop HCC (7).

Different studies were designed to examine the association between MMP-14 gene polymorphisms with the susceptibility and clinicopathological development of different cancers including small cell lung carcinoma (8), ovarian cancer (9), and HCC (10).

This study was done basically to assess the frequency of alleles of rs2236307 SNP of the MMP-14 (+7096) gene in patients with HCC and to determine if there is an association between its alleles polymorphism and the occurrence of HCC in Egyptian patients.

MATERIALS AND METHODS

Study design

A case-control study was conducted in the Tropical Medicine and Medical Biochemistry Departments, Faculty of Medicine, Zagazig University in the period between January 2019 and September 2019 with the approval of the Ethics Committee of the Faculty of Medicine (ZU-IRB#: 5143-20-1-2019). The study was conducted according to the sound clinical practice guidelines and according to the declaration of Helsinki. Written informed consent was obtained from the patients.

Patients' selection and data collection

The sample size was calculated according to the expected positive predictive value of 70, the power of the study at 85%, and a 95% confidence interval using EPI info 6. The study included 540 participants classified into three groups (180 in each group). The first group included patients with liver cirrhosis associated with HCC. The second group included patients with liver cirrhosis without HCC. The third group included healthy persons with normal liver functions. Patients who had any other tumors and patients with any acute or chronic inflammatory disorders such as systemic lupus erythematosus (SLE) or inflammatory bowel disease (IBD) were excluded from the study.

All patients underwent thorough full history, full clinical examination, complete blood count, liver function tests, kidney function tests, alpha-fetoprotein (α-FP), pelvic and abdominal ultrasonography, and triphasic computerized tomography (CT) for the assessment of hepatic focal lesion, patency of portal vein and lymph node metastasis.

MMP-14 gene polymorphism analysis in the promoter of the rs2236307 was detected by PCR-RFLP (New England Biolabs, Beverly, MA).

Statistical Analysis

All data were collected, tabulated, and statistically analyzed using SPSS software, version 20.0 for windows (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as the mean ± SD & (minimum-maximum), and qualitative data were expressed as absolute frequencies (number) & relative frequencies (percentage). Continuous data were checked for normality by using the Kolmogorov-Smirnov test. Independent samples Student's t-test was used to compare two groups of normally distributed variables while the Mann-Whitney U test was used for non-normally distributed variables. The percentage of categorical variables was compared using the Chi-square test or Fisher's exact test when appropriate. All tests were two-sided. P value < 0.05 was considered statistically significant (S), P value ≥ 0.05 was considered statistically insignificant (NS) and P value < 0.001 was considered highly statistically significant (HS).

RESULTS

As shown in Table 1, there are non-significant differences between the studied groups regarding sex and smoking, but there is a highly significant difference regarding age as older age was found among those with HCC and cirrhosis.

We found a non-significant difference between the studied groups regarding white blood cells

(WBCs) while hemoglobin level was highly significantly lower among patients with HCC compared with other groups. Regarding platelets, it was highly significantly lower among patients with cirrhosis compared with other groups (Table 2). (Editor: there are no tables attached to this article.

We found highly statistically significant differences between the studied groups regarding total bilirubin, direct bilirubin, total protein, albumin, alanine transaminase (ALT), aspartate aminotransferase (AST), creatinine, and INR. Both total, and direct bilirubin, and INR levels were highly significantly higher among patients with cirrhosis compared with other groups. ALT, AST, and creatinine levels were significantly higher among patients with HCC compared with other groups. Albumin was significantly lower among patients with cirrhosis compared with other groups (Table 3).

We found highly significant differences between the studied groups regarding CRP and ESR. Alpha-fetoprotein was significantly higher among patients with HCC with other groups (Table 4).

We found that the TC genotype was significantly higher among patients with HCC compared with the control group (Table 5).

We found that the TC genotype was higher among patients with HCC compared with patients with cirrhosis (Table 6).

We found that there were no statistically significant differences between patients with cirrhosis and the control group regarding genotypes and allele frequency. The TC genotype was higher among patients with cirrhosis compared with other groups. Having the TC genotype has almost a higher risk of five times (OR = 5.25) than having the CC genotype to have cirrhosis. Having the TT

genotype had almost a higher risk of three times (OR = 2.62) than having the CC genotype to be a cirrhotic patient (Table 7).

Table 1. Comparison of demographic data and medical history among the studied groups

Variables	HCC group (N = 180)		Cirrhotic group (N = 180)		Control group (N = 180)		F test	P value
Age: (years) Mean ± SD	55.8 ± 4.07		55.3 ± 5.68		45.8 ± 7.89		15.4	**< 0.001 (HS)**
	No	**%**	**No**	**%**	**No**	**%**	**χ^2**	**P**
Sex:								
Male	150	83.3	120	66.7	120	66.7	1.66	0.436 (NS)
Female	30	16.7	60	33.3	60	33.3		
Smoking:								
Absent:	80	44.4	80	44.4	90	50	0.149	0.928 (NS)
Present:	100	55.6	100	55.6	90	50		

F test: ANOVA. $\chi 2$: chi-square test.

Table 2. Comparison of complete blood count among the studied groups

Variables	HCC group (N=18)	Cirrhotic group (N=18)	Control group (N=18)	F test	P value	LSD
Hemoglobin: Mean±SD g/dl	9.56±1.53	9.62±0.86	12.4±1.83	**22.7**	**<0.001 (HS)**	>0.05 [1] <0.05 [2] <0.05 [3]
WBCs: Mean±SD×10^3/ml	7.26±1.40	7.58±1.52	7.20±1.91	0.288	0.751 (NS)	>0.05 [1] >0.05 [2] >0.05 [3]
Platelets: Mean±SD×10^3/ml	99.4±20.5	77.6±14.4	354.2±47.2	**447**	**<0.001 (HS)**	>0.05 [1] **<0.05** [2] **<0.05** [3]

F test: ANOVA. LSD: Least-significant difference post-hoc test
LSD1: HCC group versus cirrhotic group.
LSD2: HCC group versus control group.
LSD3: cirrhotic group versus control group.

Table 3. Comparison of liver, kidney functions and INR among the studied groups

Variables	HCC group (N=18)	Cirrhotic group (N=18)	Control group (N=18)	F test	P value	LSD
Total bilirubin: Mean±SD mg/dl	3.58±0.87	4.08±0.90	1.06±0.15	89.01	**<0.001 (HS)**	>0.05 [1] <0.05 [2] <0.05 [3]
Direct bilirubin: Mean±SD mg/dl	2.5±0.50	2.9±0.40	0.24±0.05	269	**<0.001 (HS)**	>0.05 [1] <0.05 [2] <0.05 [3]
Total protein: Mean±SD g/dl	5.96±0.41	6.02±0.56	6.62±0.32	12.10	**<0.001 (HS)**	>0.05 [1] <0.05 [2] <0.05 [3]
Albumin: Mean±SD g/dl	2.59±0.54	2.51±0.48	3.76±1.30	11.95	**<0.001 (HS)**	>0.05 [1] <0.05 [2] <0.05 [3]
ALT: Mean±SD Iu/L	58.7±13.4	37.5±9.5	22.6±6.48	57.18	**<0.001 (HS)**	>0.05 [1] <0.05 [2] >0.05 [3]
AST: Mean±SD Iu/L	66.5±12.5	42.7±9.70	19.1±4.40	112	**<0.001 (HS)**	<0.05 [1] <0.05 [2] <0.05 [3]
Creatinin: Mean±SD mg/dl	1.22±0.49	1.15±0.57	0.80±0.27	4.32	**0.01 (S)**	>0.05 [1] <0.05 [2] <0.05 [3]
INR: Mean±SD	1.29±0.26	1.46±0.18	1.07±0.12	17.52	**<0.001 (HS)**	<0.05 [1] <0.05 [2] <0.05 [3]

F test: ANOVA.
LSD: Least-significant difference post-hoc test

We found that there were no statistically significant differences between different genotypes of HCC patients regarding focal lesion number and size, treatment outcome, and site of focal lesions (Table 8).

We found that metastatic lesions and portal vein thrombosis (PVT) were significantly higher among the TC

Table 4. Comparison of CRP, ESR and alpha feto protein among the studied groups

Variables	HCC group (N=18)	Cirrhotic group (N=18)	Control group (N=18)	F test	P value	LSD
CRP: Mean±SD mg/l	18.1±2.41	24.6±4.34	5.88±1.32	184	**<0.001 (HS)**	>0.05 [1] <0.05 [2] <0.05 [3]
ESR: Mean±SD mm	48.2±8.9	17.3±3.5	5.11±1.39	285	**<0.001 (HS)**	<0.05 [1] <0.05 [2] <0.05 [3]
AFP Mean±SD *ng/ml*	1097±212.5	19.8±6.8	1.9±1.62	467	**<0.001 (HS)**	<0.05 [1] <0.05 [2] >0.05 [3]

F test: ANOVA.
LSD: Least-significant difference post-hoc test

Table 5. Studying of genotype and alleles in between the control and HCC groups:

Variables	Control group (n=180)		HCC group (n=180)		OR (95% CI)	P
	No.	%	No.	%		
Genotype:						
TT:	60	33.3	30	16.7	4.5 (0.37-54.1)	**0.007**
TC:	30	16.7	140	77.8	**42 (3.76-469)**	**(S)**
CC:	90	50	10	5.6	Reference	
Alleles:						
T:	150	41.7	200	55.6	1.75 (0.68-4.45)	0.239
C:	210	58.3	160	44.4	Reference	(NS)

CI: confidence interval.
OR: odds ratio.

Table 6. Studying of genotype and alleles in between the cirrhotic and HCC groups

Variables	Cirrhotic group (n=180)		HCC group (n=180)		OR (95% CI)	P
	No.	%	No.	%		
Genotype:						
TT:	70	38.9	30	16.7	1.7 (0.13-22.5)	**0.04**
TC:	70	38.9	140	77.8	8 (0.74-85.7)	**(S)**
CC:	40	22.2	10	5.6	Reference	
Alleles:						
T:	210	58.3	200	55.6	1.89 (0.35-2.27)	0.811
C:	150	41.7	160	44.4	Reference	(NS)

OR: odds ratio.
CI: confidence interval.

group compared with the TT and the CC groups (Table 9), so we found that rs2236307polymorphism increases the risk of HCC.

DISCUSSION

In Egypt, as a result of the HCV epidemic HCC has become the most common cancer among Egyptians with late presentation and a high recurrence rate after treatment (11).

Despite chronic infection with HBV or HCV as the most well-established risk factors for HCC during their lifetime only a fraction of infected patients develop HCC, which suggests that gene polymorphism and protein

Table 7. Studying of genotype and alleles in between the control and cirrhotic groups

Variables	Control group (n=180)		Cirrhotic group (n=180)		OR (95% CI)	P
	No.	%	No.	%		
Genotype:						
TT:	6	33.3	7	38.9	2.62 (0.52-13.07)	0.165
TC:	3	16.7	7	38.9	5.25 (0.87-31.5)	(NS)
CC:	9	50	4	22.2	Reference	
Alleles:						
T:	15	41.7	21	58.3	1.96 (0.76-5.02)	0.811
C:	21	58.3	15	41.7	Reference	(NS)

OR: odds ratio.
CI: confidence interval

Table 8. Studying of different tumor characters in different genotypes among the HCC patient's group

Variables	TT group (n=3)		TC group (n=14)		CC group (n=1)		χ^2	P
	No	%	No	%	No	%		
Number of focal lesions								
1:	2	66.7	5	35.7	1	100	2.954	0.858 (NS)
2-3:	0	0	2	14.3	0	0		
>3:	1	33.3	7	50	0	0		
Focal lesion size:								
<3 cm:	2	66.7	3	21.4	0	0	4.554	0.336 (NS)
3-5 cm:	1	33.3	4	28.6	0	0		
>5 cm:	0	0	7	50	1	100		
Treatment outcome:								
Death:	1	33.3	6	42.9	0	0	6.612	0.579 (NS)
Well ablated:	2	66.7	2	14.3	1	100		
New focal lesion:	0	0	1	7.1	0	0		
Local recurrence:	0	0	2	14.3	0	0		
Decompensation:	0	0	3	21.4	0	0		
Site of focal lesion:								
Left lobe:	0	0	2	14.3	0	0	4.114	0.391 (NS)
Right lobe:	3	100	6	42.9	1	100		
Both lobes:	0	0	6	42.9	0	0		

Table 9. Studying of invasion parameters in different genotypes among the HCC patient's group

Variables	TT group (n=30)		TC group (n=140)		CC group (n=10)		χ^2	P
	No	%	No	%	No	%		
Metastatic lesions:								
Absent:	18	60	113	80.8	9	90	**7.049**	**0.029**
Present:	12	40	27	19.2	1	10		**(S)**
PVT:								
Absent:	20	66.7	71	50.7	9	90	**7.63**	**0.022**
Present:	10	33.3	69	49.3	1	10		**(S)**

expression play a key role in determining susceptibility and occurrence of HCC. Numerous candidate gene studies have reported associations between SNPs and the presence of HCC (12).

MMP-14 is one of the pericellular collagenases to degrade extracellular matrix (ECM), which is involved in the modulation of susceptibility or clinicopathological features of cancer. The contributions of MMP-14 on the susceptibility or clinicopathological features in HCC have been well documented, and the expression of MMP-14 in HCC has also been observed (13).

In our study age was highly significant between the three groups. These results are in agreement with Shaker and colleagues (14) who found that the most frequent age category affected by HCC was 51–60 years.

Since MMP-14 activates proMMP2 on the cell surface in the presence of a low concentration of TIMP2, it is suggested that the MMP2/MMP-14/TIMP2 system plays a significant role in the MMP-mediated degradation of the ECM. Therefore, polymorphisms of the MMP-14 gene may be responsible for the up-regulation of the protein activity leading to the activation of proMMP2, which plays a critical role in HCC invasion and metastasis (15).

Our study shows that the CC genotype is lower in the HCC group in comparison with the control group in agreement with Chen and colleagues (10) who demonstrated that rs2236307C/C genotypes had a lower risk for HCC compared with their corresponding wild-type genotypes.+7096 C/C allele might reduce the bioactivity or expression of MMP-14, and the cellular behaviors are thereby aberrant. The ratiocination provides a reasonable explanation for the low risk of having HCC of these two rs2236307 alleles.

In our study, we found that the TC genotype was significantly higher among HCC patients compared with the control group. Having the TC genotype has almost a higher risk of 42 times (OR=42) than having the CC genotype to develop HCC in agreement with Chen and colleagues (10). Also, we found that the TC genotype was significantly higher among HCC patients compared with the cirrhotic group. Having the TC genotype has almost a higher risk of 8 times (OR=8) than having the CC genotype to develop HCC. Also, we found that metastatic lesions and portal vein thrombosis (PVT) were significantly higher among the TC group compared with the TT and the CC groups. This may be explained by the fact that the+7096 T/C (Gly285Gly) polymorphisms might be able to change the translational rate of mRNA stability of the MMP-14 protein (16).

Also, rs2236307 polymorphism was found to increase the risk of squamous cell neoplasia of the uterine cervix (17), COPD (18), and carotid plaque formation (19).

CONCLUSION

The polymorphisms of rs2236307 increase the risk of HCC. Both TC and CC genotypes showed HCC risk association but the the CC genotype appeared with a lower potential.

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