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Possible Relationship between Matrix Metalloproteinases Genotyping and Risk of Hepatocellular Carcinoma in HCV Infected Patients

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Authors' contributions

Authors NR, HM and WE carried out the molecular genetic studies, participated in the primer sequence alignment, RFLP, real time PCR and drafted the manuscript. Author KMEG carried out CT scanning to diagnose HCC. Author SMG supplied the patients' samples. Authors YE, NR and KMEG participated in the design of the study and performed the statistical analysis. Author SMG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The annual number of new cases of hepatocellular carcinoma (HCC) worldwide is over 1 million. In developing countries, the major cause of HCC is chronic hepatitis C virus (HCV) infection. Various studies have reported an association between functional gene polymorphism of matrix metalloproteinases (MMP) promoters and different cancers.
Rationale: This study examined the association between MMP1 -1607, MMP9-1562, MMP14-6727

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and MMP14-6767 gene polymorphisms and risk of HCC in HCV infected patients.
Methods: The study enrolled 160 HCC patients, 91 with & 69 without chronic HCV infection, and 140 healthy subjects as control group. Genomic DNA was analysed for MMPs gene polymorphism using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) for MMP1 and MMP9 but real time PCR was used for MMP14 genotyping.
Results: MMP1-2G allele carriers had higher susceptibility of developing HCC in HCV infected patients. MMP9-1562 T/T genotype had high risk of developing HCC in HCV and non HCV related patients when compared to healthy controls. A significant lower risk for HCC was shown in individuals with MMP14-6767 G/A. The distribution frequency of MMP14-6767 G and MMP14-6727 C allele and homozygote genotype was significantly higher in HCC patients.
Conclusion: MMP-1 -1607 2G allele carriers would alter the risk of HCC under specific conditions such as chronic infection with HCV. People with MMP9-1562 T/T genotype are at risk of developing HCC. MMP14-6767 G and MMP14-6727 C allele carriers and homozygote genotype might contribute to the prediction of susceptibility and pathological development of HCC in HCV infected patients.

Keywords: Metalloproteinases; gene polymorphism; HCV; risk of HCC.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is considered as one of the most prevalent tumors with high incidence and mortality rates. HCC is the 6th most common cancer worldwide and the 3rd leading cause of cancer-related death [1]. More than 80% of HCCs develop in Asian and African countries. In Africa, especially in Egypt, hepatitis C virus (HCV) is the most leading cause of the increased HCC incidence [2]. Egypt is an endemic area of HCV. Globally, HCV infection represents 2% of people, while around 14% of Egyptian population gives positive test results for HCV antibodies. Nevertheless, approximately 10% of people carry HCV viral RNA and are consequently chronically infected [3].

Cirrhosis and HCC are the most serious complications of chronic HCV infection [4]. Donato et al. noted that the risk of HCC increased 17-fold among HCV-infected patients compared with anti-HCV negative controls. The exact pathogenesis of hepatic injury is not well understood in HCC [5]. It has been suggested that HCV induces cytotoxic $CD8^+$ T cells to elicit an immune response against HCV-infected hepatocytes leading to injury of liver cells in terms of fibrosis or cirrhosis and even HCC development [6].

Matrix metalloproteinases (MMPs) are, zinc dependent proteolytic enzymes, that cleave extracellular matrix (ECM) proteins and non-matrix substrates as well. The genes family of MMPs consists of 24 human endopeptidases that collectively can degrade all protein components of the ECM, thereby facilitating tissue remodeling and cell migration. These enzymes can also cleave and activate growth factors and cytokines, take out their receptors from the cell surface, and disrupt the interaction of growth factors and receptors with associated proteins [7]

MMPs have important functions in pathologic conditions that are characterized by the excessive degradation of ECM, such as tumor metastasis, rheumatoid arthritis and periodontal disease [8]. MMPs play an important role in several steps of cancer development through angiogenesis, apoptosis inhibition, cell cycle regulation, cell differentiation or cancer metastasis [9].

Several polymorphisms in the promoters of a number of MMP genes, which are thought to affect the respective MMP production in an allele-specific manner, have been well characterized. There is increasing evidence indicating that these functional polymorphisms may contribute to inter-individual differences in susceptibility to a wide spectrum of cancers [10,11]. Moreover, gene polymorphisms in the promoter regions of MMP genes, including MMP-1, MMP-3 and MMP-9 were found [12].

MMP1, also called interstitial collagenase-1, and MMP3, also called stromelysine-1, are two important members in MMPs family. They are located on 11q22 with 11 exons and 10 introns and play important roles in cancer development and metastasis. MMP-1 is one of the major proteinases that have proteolytic activity against collagens which is the most abundant ECM proteins in liver fibrosis [13]. MMP1 and MMP3 gene promoters are polymorphic and those

polymorphisms may affect transcription of MMP genes. MMP1 promoter region contains a guanine insertion/deletion polymorphism at position -1607, with one allele having a single guanine nucleotide (1G) and the other having two (2G) [14].

MMP-9, also called gelatinase B, is the most complex member of MMP family. It is produced by macrophages and has proteolytic activity against major components of the basement membrane like collagen type IV & V, gelatin, fibronectin and elastin. MMP9 is responsible for extravasation, migration and tissue remodeling during chronic inflammation. The expression of MMP9 is up-regulated in various human cancers. The C to T substitution at position 1562 in the promoter region of MMP9 gene has a higher transcriptional activity of the T-allelic promoter. This might be caused by DNA protein interaction abolishment by the C to T substitution at this polymorphism site [12].

MMP14, also known as membrane type 1 matrix metalloproteinase, helps modify and breakdown various components of the extracellular matrix forming a pericellular collagenase against ECM. In different cell types, MMP14 acts on their surrounding environment inducing tissue remodeling, angiogenesis, invasion and metastasis by catalyzing pericellular collagenolysis [15]. Chen et al. found that both MMP14 -6767 G/A and 7096 C/C exhibited a significant lower risk to have HCC. They also revealed that the distribution frequency of 165 T: 221 T: 6727 C: 6767 G: 7096 T: 8153 G haplotype and diplotype was significantly higher in the HCC patients than the healthy control subjects [16].

Comparing genetic information of HCC patients with control subjects and with HCV infected patients is particularly valuable to mark a target gene for predicting susceptibility and pathological development of HCC. This study aimed at evaluation of the association between MMP-1-1607 1G/2G, MMP-9-1562 C/T, MMP-14-6767 G/A and MMP-14-6727 C/G gene polymorphisms and the susceptibility of HCC in HCV infected patients in Egyptian population.

2. SUBJECTS AND METHODS

2.1 Study Design

The subjects of this study were divided into 3 groups, group I: 140 healthy persons as control group (mean age 64±2.3, 73 males and 67 females), group II: 91 patients with HCV related HCC (mean age 63.0±5.5, 51 males and 40 females) and group III: 69 non HCV related HCC (mean age 65±6.9, 38 males and 31 females). Patients of group II were all positive for serum HCV RNA. All the participants were recruited from Zagazig Univeristy Hospitals during the period from October 2012 to December 2014.

2.2 Participants

The patients were diagnosed with HCC according to the characteristic criteria of the national guidelines for HCC, such as liver injury diagnosed by histology or cytology irrespective of a-fetoprotein titer where imaging data (at radio-diagnosis department) showed one of the following three conditions: [1] one or more liver masses ≥ 2 cm in diameter via both computed tomography (128 slice Philips, Netherlands) and magnetic resonance imaging (1.5 T Philips, Netherlands); [2] one imaging data with early enhancement and a high level of AFP ≥ 400 ng/ml; [3] one imaging data with early arterial phase contrast enhancement plus early venous phase contrast washout regardless of AFP level [17]. The study was approved by the ethical committee of Faculty of Medicine, Zagazig University and written informed consent was obtained from all participants.

For all participants, serum alanine transaminase (ALT) and aspartate transaminase (AST) (Elitech), serum tumor marker alpha fetoprotein (AFP) by ELISA (Equipar, Saronno, Italy) were measured.

2.3 DNA Extraction

Genomic DNA was isolated and purified from 200 µl whole blood (EDTA) using the protocol provided by the manufacturer (Qiagen GmbH, Hilden, Germany). The purified DNA was safely stored at −20℃ for later use.

2.4 Genotype Analysis of MMP-1-1607 and MMP-9-1562 Gene Polymorphisms

MMP1-1607 1G/2G (rs1799750) and MMP9-1562 C/T (rs3918242) gene polymorphisms were analyzed using polymerase chain reaction followed by the restriction fragment length polymorphism (PCR-RFLP) method according to Okamoto et al. [18]. Polymerase chain reaction (PCR) was performed in a final volume of 50 µl containing 10 µl (~50ng) of genomic DNA, 1 µl of

each primer (0.5 µM), 25 µl of 2x Super Hot PCR Master Mix and 13 µl deionized water. The amplification was carried out using thermal cycler 480, Perkin Elmer (Norwalk, CT 06856, USA). The PCR products were digested with 5 U of the restriction enzyme listed in Table 1 at 37Cº for 3 h (Fast Digest, Thermoscientific), and the amplified products were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide using Submarine Gel Electrophoresis System (Pharmacia Biotech by SEMKO AB, Sweden) and submarine chamber (Maxicell, EC360, M-E-C apparatus Co.St Petersburg, Florida, USA). The gel was visualized under a UV transilluminator with Gene Ruler^{Tm} 100 base pair DNA ladder (Fermentas Canada Inc, Burlington, Ontario, Canada). Primers sequence, PCR protocol, restriction enzymes and length of expected bands for MMP1-1607 1G/2G and MMP9-1562 C/T gene polymorphisms are shown in Table 1.

2.5 Allele Discrimination of MMP-14-6727 and MMP-14-6767 by Real-Time PCR

The allelic discrimination of the MMP14-6727 (rs2236302) and MMP14-6767 (rs1042704) gene polymorphisms were assessed using TaqMan assay (Applied Biosystems) with the Stratagene, MX3000P quantitative PCR System (Agilent technologies) and analyzed using MxPro QPCR Software (Agilent technologies). Table 2 lists the primers and probe sequences for each polymorphism. Primers were designed according to NCBI PRIMER-BLAST software (Washington, USA) from www.ncbi.nlm.nih.gov/tools/primer-blast. The final volume for each reaction was 25 µl, containing 12.5 µl 2X TaqMan Universal Master Mix (Applied Biosystems), 0.5 µM TaqMan probes, 1 µM of each primer 4.5 µl deionized H_2O and 5 µl genomic DNA. The real time PCR reaction included pre-incubation at 50ºC for 2 min and initial denaturation step at 95ºC for 10 min, followed by 45 cycles, each consisting of 95ºC for 30 s and 60ºC for 1 min.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS version 11 (Chicago, IL, USA) statistical package. Continuous variables were expressed as means ± SD. Two-tailed t test was used for continuous variables, and Chi-square (χ^2) test for categorized variables. Hardy-Weinberg equilibrium (HWE) analysis was performed to compare observed and expected genotype frequencies using χ^2 test. In addition, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated as a measure of the association of the MMP1, 9 an 14 polymorphisms with HCC development. A difference was considered significant if *p* was <0.05.

3. RESULTS

3.1 Baseline Characteristics of the Studied Groups

There were insignificant difference as regards both age and sex (*p*>0.05) between HCC patients and controls. In the HCC groups, there was a significant difference of the mean values of serum ALT, AST and AFP levels (P<0.05) in relation to control group (Table 3).

In our recruited groups, the genotype frequencies of all analyzed polymorphisms were conformed to the Hardy–Weinberg equilibrium (P>0.05). Table 4 shows the genotypes distribution and allele frequencies in MMP1-1607 (1G/2G), MMP9-1562 (C/T), MMP14-6727 C/G and MMP14- 6767 G/A in HCV and non-HCV related HCC patients as compared to healthy control.

3.2 Genotype and Allele Distributions of MMP1- 1607 and MMP9-1562 Gene Polymorphisms

For MMP1- 1607 (1G/2G) (Fig. 1), significant higher representation of the 2G/2G genotype and 2G allele frequency was found in the HCV related HCC group (P= 0.03 and P= 0.01 respectively) and not in non HCV related HCC when compared to healthy control group. HCC risk in 2G/2G genotype is 1.91-fold (95%CI, 1.06-3.42) and is 1.62-fold (95%CI, 1.11-2.35) in 2G carriers in HCV related HCC when compared to healthy individuals. There was no significant difference in representation of 1G genotype between different studied groups.

RFLP-PCR genotyping of 1562 C/T in the promoter of MMP-9 gene (Fig. 2) revealed a high significant increase in the TT genotype frequency and T allele frequency in patients with HCV related HCC as compared to that in normal individuals (P<0.001). In addition, there was a significant increase in TT genotype frequency and T allele frequency in overall HCC patients when compared to normal individuals (P=0.001 and P<0.001respectively). However, there was insignificant difference in the genotype distribution between non HCV related HCC

patients and normal individuals (P>0.05). A significant higher representation of the MMP9-1562 T/T genotype and T allele frequency was found between HCV related HCC group as compared to non HCV related HCC group (P= 0.04 and P= 0.01 respectively). The risk of HCC development in MMP9-TT genotype was 3.14-fold (95%CI, 1.76-5.61) in HCV infected patients as compared to healthy control and was 2.01-fold (95% CI, 1.03–3.90) as compared to non HCV related HCC (Table 4).

Real time allele discrimination results revealed significant high distribution of MMP14-6727 C and MMP14-6767 G alleles and homozygous genotypes in both HCV related HCC patients (P<0.05) and in overall HCC patients (P<0.05) when compared to healthy individuals. OR and its 95% CI of MMP14-6727 C/C and MMP14-6767 G/G, 2.7 (1.26-5.77) and 5.18 (1.50-17.90) respectively, showed a higher risk for development of HCC in HCV infected patients and overall HCC patients. However, MMP14-6727 G and MMP14-6767 A allele frequencies exhibited significant lower risk of having HCC compared with their corresponding wild-type allele in HCV related HCC patients (P= 0.008 and P= 0.004 respectively) and in overall HCC patients (P= 0.01 and P= 0.02 respectively) (Table 4).

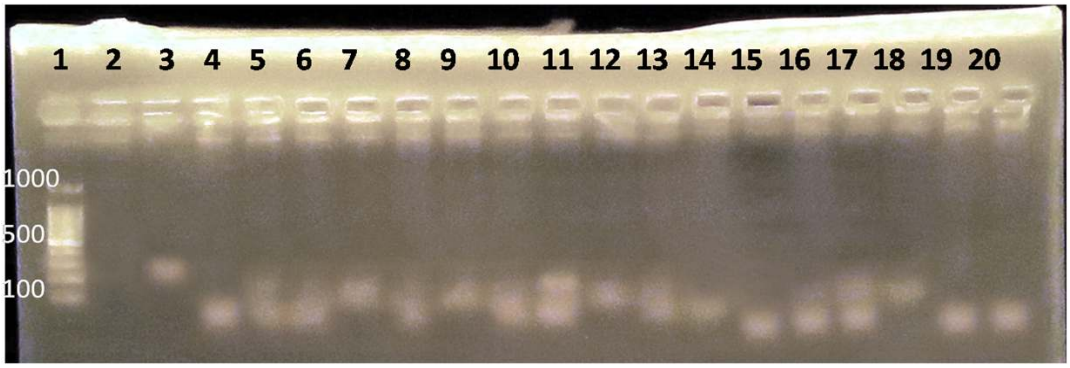

Fig. 1. Agarose gel electrophoresis picture for RFLP- PCR products of MMP1 gene showing DNA marker in lane 1, negative control in lane 2. Lanes 3 show PCR product (234bp) of MMP1 gene (before digestion). Other lanes show digested PCR product with *XmnI* restriction enzyme. 2G/2G genotype (117bp) is shown in lanes 7, 9, 12, 13, 14 and 18. Lanes 5, 6, 11, 16 and 17 show 1G/2G genotype (117bp and 89bp). Genotype 1G/1G (89bp) is shown in lanes 4, 15, 19 and 20

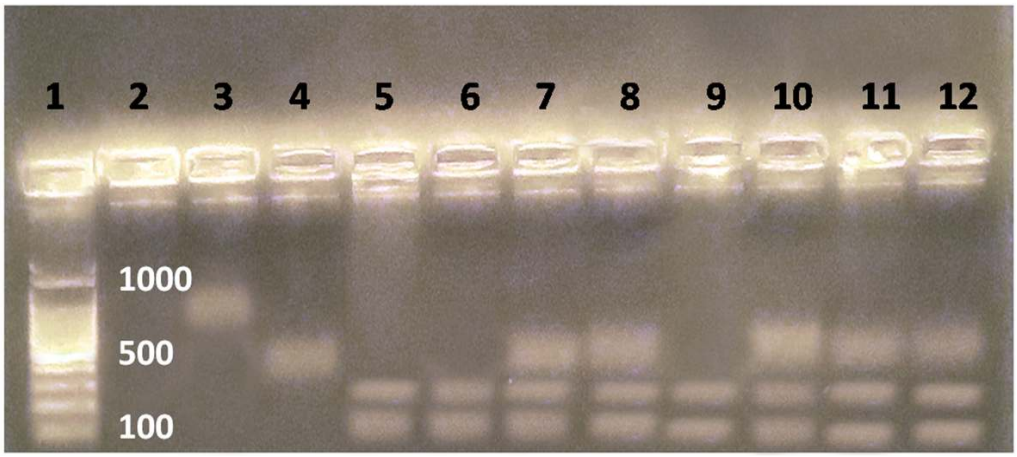

Fig. 2. Agarose gel electrophoresis picture for RFLP-OCR products of MMP9 showing DNA marker in lane 1, negative control in lane 2. Lanes 3 show PCR product (870bp) of MMP9 gene (before digestion). Other lanes show digested PCR product with *SphI* restriction enzyme. C/C genotype (435bp) is shown in lane 4. Lanes 5, 6 and 9 show T/T genotype (247bp and 188bp). Genotype C/T (435bp, 247bp and 188bp) is shown in lanes 7, 8, 10, 11 and 12

Table 1. The primers sequence, PCR protocol, restriction enzymes, and length of products for genotyping of MMP1- 1607 (1G/2G), and MMP9-1562 (C/T) gene polymorphisms

	Primer sequence (5'-3')	PCR protocol	Length of PCR product	Restriction Enzyme	Length of digested products
MMP1-1607 (1G/2G) rs; 1799750 [19]	F: TCGTGAGAATGTCTTCCCATT R: CTTGGATTGATTTGAGATAAGTGAAATC	-1 cycle at 95 ˚C for 1 min. -35 cycles at 95 ˚C for 1 min, 55 ˚C for 30 sec and 72 ˚C for 30 sec. -1 cycle at 72 ˚C for 5 min.	234 bp	*Xmn*I	2G allele: 117 bp 1G allele: 89&28 bp
MMP9-1562 (C/T) rs; 3918242 [20]	F: GCCTGGCACATAGTAGGCCC- R: CTTCCTAGCCAGCCGGCATC	-1 cycle at 95 ˚C for 2 min. -35 cycles at 95 ˚C for 45 sec, 67 ˚C for 45 sec and 72 ˚C for 45 sec. -1 cycle at 72 ˚C for 5 min.	870 bp	*Sph*I	C allele: 435 bp T allele: 247&188 bp

Table 2. The primers sequence, probe sequence, PCR protocol and reference SNP of MMP14-6727 C/G and MMP14-6767 G/A gene polymorphisms

	Primer sequence (5'-3')	Probe sequence (5'-3')	PCR protocol
MMP14-6727 (C/G) rs; 2236302 [21]	F: GAGCATTCCAGTGACCCCTC R: GGGCAGCACAAAATTCTCCG	Probe-G: VIC- GTAAAACGGTGCCA Probe-C: FAM- GTAAAAGGGTGCCA	Pre-incubation at 50ºC for 2 min and initial denaturation step at 95ºC for 10 min, followed by 45 cycles, each consisting of 95ºC for 30 s and 60ºC for 1 min.
MMP14-6767 (G/A) rs; 1042704 [21]	F: GGAGAATTTTGTGCTGCCCG R: GGGTGTAGACTACTCGCCATA	Probe-A:VIC- CATCATTGGGCAGCA Probe-G:FAM- CATCATCGGGCAGCA	

Table 3. Demographic characteristics and clinicopathological parameters of the studied groups

Parameters	HCV related HCC (*n* = 91)	*P* value	Non HCV related HCC (*n* = 69)	*P* value	Control group (*n* = 140)
Age (years)	63±5.5	0.06	65±6.9	0.12	64±2.3
Sex (male/female)	51 (56%) / 40(44%)	0.56*	38 (55%) / 31 (45%)	0.69*	73 (52.1%) /67 (47.9%)
Alanine Transaminase (IU/L)	62±5	< 0.001	61±1.1	< 0.001	22±6
Aspartate Transaminase (IU/L)	85±9	< 0.001	71±8	< 0.001	24±9
AFP (ng/mL)	486±394 <400 (12%) ≥400 (88%)	< 0.001	463±287 <400 (15%) ≥400 (85%)	< 0.001	11±2.2
Tumor size (cm)	3.6±2.3	------	2.4±1.1	-------	0

**p value is calculated versus control group*

Table 4. Genotype and allele frequencies of MMPs gene polymorphisms in patients with hepatocellular carcinoma and controls and OR for hepatocellular carcinoma risk

Polymorphism	Controls n, (%)	HCV related HCC n, (%)	OR (95%CI)	P	Non HCV related HCC n, (%)	OR (95%CI)	P	Overall individual cases n, (%)	OR (95%CI)	P
MMP1 – 1607										
1G/1G	46 (32.9)	20(21.9)	0.58(0.31-1.06)	0.07	22 (31.9)	0.96(0.52-1.77)	0.89	42(26.2)	0.73(0.44-1.1.20)	0.21
1G/2G	63 (45.0)	39(42.9)	0.92(0.84-1.56)	0.75	30 (43.5)	0.94(0.53-1.68)	0.84	69(43.1)	0.93(0.59-1.46)	0.74
2G/2G	31(22.1)	32(35.2)	1.91(1.06-3.42)	0.03	17 (24.6)	1.15(0.58-2.26)	0.69	49(30.7)	1.55(0.92-2.62)	0.10
1G allele frequency	155 (55.4)	79 (43.4)	Reference	-------	74 (54.0)	Reference	-----	153(47.8)	Reference	-----
2G allele frequency	125 (44.6)	103(56.6)[a]	1.62(1.11-2.35)	0.01	64 (46.0)	1.07(0.71-1.61)	0.74	167(52.2)	1.35(0.98-1.87)	0.07
MMP9 – 1562										
C/C	51 (36.4)	12 (13.2)	0.27(0.13-0.53)	<0.001	17 (24.6)	0.57(0.30-1.10)	0.09	29 (18.1)	0.39(0.23-0.66)	0.0003
C/T	60 (42.9)	38 (41.7)	0.96(0.56-1.63)	0.86	32 (46.4)	1.15(0.66-2.06)	0.63	70(43.8)	1.04(0.66-1.64)	0.89
T/T	29 (20.7)	41 (45.1)[b]	3.14(1.76-5.61)	<0.001	20(29.0)	1.56(0.81-3.03)	0.18	61 (38.1)	2.36(1.40-3.96)	0.001
C allele frequency	162(57.9)	62(34.1)	Reference	----------	66 (47.8)	Reference	------	128(40.0)	Reference	-------
T allele frequency	118(42.1)	120 (65.9)[c]	2.66(1.80-3.92)	<0.001	72 (52.2)	1.45(0.99-2.26)	0.05	192(60.0)	2.06(1.49-2.85)	<0.001
MMP14 +6727										
C/C	105(75.0)	81(89.0)	2.7(1.26-5.77)	0.009	57(82.7)	1.58(0.76-3.29)	0.21	138(86.2)	2.09(1.16-3.77)	0.013
C/G	31(22.1)	9(10.0)	0.38(0.17-0.86)	0.02	11(15.9)	0.67(0.31-1.42)	0.29	20(12.5)	0.50(0.27-0.93)	0.03
G/G	4(2.9)	1(1.0)	0.38(0.04-3.43)	0.65	1(1.4)	0.50(0.05-4.56)	0.67	2(1.3)	0.43(0.08-2.39)	0.42
C allele frequency	241(86.1)	171(94.0)	Reference	--------	125(90.6)	Reference	------	296(92.5)	Reference	-------
G allele frequency	39(13.9)	11(6.0)	0.34(0.20-0.79)	0.008	13(9.4)	0.64(0.33-1.23)	0.19	24(7.5)	0.50(0.29-0.86)	0.01
MMP14 +6767										
G/G	119(85.0)	88(96.7)	5.18(1.50-17.90)	0.004	62(89.9)	1.56(0.63-3.88)	0.33	150(93.8)	2.65(1.20-5.84)	0.01
G/A	20(14.3)	3(3.3)	0.20(0.06-0.71)	0.006	6(8.7)	0.57(0.22-1.49)	0.23	9(5.6)	0.36(0.16-0.81)	0.011
A/A	1(0.7)	0(0)	----------	------	1(1.4)	2.04(0.12-33.18)	1	1(0.6)	0.87(0.05-14.11)	1
G allele frequency	258(92.1)	179(98.4)	Reference	--------	130(94.2)	Reference	------	309(96.6)	Reference	------
A allele frequency	22(7.9)	3(1.6)[d]	0.20(0.06-0.67)	0.004	8(5.8)	0.72(0.31-1.67)	0.44	11(3.4)	0.42(0.20-0.88)	0.02

[a]p= 0.07; OR=1.51; 95% CI (0.97–2.35); versus non HCV–related HCC
[b]p= 0.04; OR=2.01; 95% CI (1.03–3.90); versus non HCV –related HCC
[c]p= 0.01; OR=1.77; 95% CI (1.13–2.79); versus non HCV –related HCC
[d]p= 0.06; OR=0.27; 95% CI (0.07–1.05); versus non HCV –related HCC

4. DISCUSSION

Various studies have suggested strong association between MMPs gene polymorphism and the risk of different cancers development. Our study is one of few researches that examined the association between MMP-1, 9 and 14 gene polymorphisms and risk of developing HCC in HCV infected patients in Egypt (*4, 6, and 1*8). Hepatitis virus infection is related to the enhanced oxidative stress in liver cells and results in DNA instability, hence, increasing the risk of developing cirrhosis and/or HCC [22]

Our work reported that the frequency of MMP-1-1607 2G allele carriers and homozygotes genotype was significantly increased in HCC patients with HCV as compared to healthy controls. This agrees with what Okamoto et al [13] and Zhai et al. [23] who studied Japanese and Chinese populations, respectively.

The insertion of a G nucleotide at 1607 bp in the nucleotide sequence of the MMP-1 gene promoter generates a new 5-GGA-3 sequence that corresponds to a core recognition sequence of the binding site for members of the Ets family of transcription factors [24,25]. The 2G homozygous polymorphism of the promoters results in an increased transcription activity in melanoma cell lines and in normal fibroblasts compared with 1G homozygotes and controls. Similarly, an association between local MMP-1 overexpression and unfavorable prognosis has been reported for both CRC and esophageal cancer [26,27].

However, Peng et al. reported that a significant association between MMP-1 1G/2G polymorphism and cancer risk in their meta-analysis which did not include hepatic cancer. They suggested that the MMP-1 -1607 1G/2G might play different roles in different types of cancers. One factor contributing to this discrepancy is that different human tissues might have different expression profiles of MMP-1 and transcription factors, and thus the same polymorphism might play different parts in different tissues. Another factor is that different cancers may be influenced by different environmental factors, which may support or decline the association with promoter polymorphisms of MMP-1 [*2*8]. It would be expected that the MMP genotypes would alter the risk of HCC under specific conditions such as exposure to cigarette smoke and alcoholic consumption. In fact, many factors such as chronic infection with HBV, male gender, family history, smoking, and alcoholic consumption have been shown as self-regulating risk factors for HCC [29,30]. HCV infection might be a contributing factor for the association of 2G allele with HCC development.

In a global analysis, the results showed a significant association between MMP1 1G/2G polymorphism and cancer risk in recessive genetic model, dominant genetic model and allelic contrast. The same trend of associations was also observed in colorectal cancer, head and neck cancer and renal cancer [28]

We also observed a significant relationship between MMP-9 -1562 T/T genotypes and T allele carriers with the development of HCC in overall cases and in HCV infected patients but there was no significant difference in the MMP-9 TT genotype and the T allele between HCC patients without HCV viral hepatitis when compared to healthy subjects. However, Okamoto et al., found a positive relationship between MMP-9 T carriers and poor HCC differentiation, but could not demonstrate a significant relationship with the development of HCC [17]. Matsumura et al. found significant associations between this polymorphism and the invasive phenotype of gastric cancer [31].

Angiogenesis is a very important step in cancer progression because it promotes both primary tumor growth and metastasis dissemination MMP-9 cleaves plasminogen to generate angiostatin, one of the most potent inhibitors of angiogenesis [32]. HCC is a highly vascular solid tumor in which angiogenesis plays an important role, therefore raising the hypothesis that the 2 main MMP-9 functions, the degradation of the ECM and angiogenesis inhibition, induce a complicated transition in the balance in HCV-related HCC [18].

Zhang et al. reported that MMP-9 expression levels could be influenced by genetic variation in MMP-9 gene promoters. The sequences in the promoter region containing the MMP9-1562 C/T polymorphic site constitute a chief regulatory element which appears to be a binding site for a transcription repressor protein. Therefore, C/T substitution at the polymorphic site eliminates the DNA-protein interaction, resulting in a higher activity of the T-allelic promoter [33]. Transient transfection and DNA-protein interaction assays indicated that T allele-associated promoter activity (due to the preferential binding of a

putative transcriptional repressor protein) was higher than the C allele-associated promoter activity [10].

The prevalence of the MMP-9 C allele was found to be significantly greater in liver cirrhosis patients than in chronic hepatitis patients, suggesting that the MMP-9 C/C genotype and C allele carriers may contribute to the progression of liver fibrosis. Hence, MMP-9 CC genotype with low transcriptional activity has lower production of MMP-9 in the fibrotic liver, resulting in rapid progression of liver fibrosis due to decreased degradation of the ECM [12].

MMP9 -1562 C/T polymorphisms were significantly associated with lung cancer risk among Asian population [34] and bladder cancer susceptibility [35].

MMP-14, which initiates liver metastasis, is over-expressed in HCC [36]. Our results revealed that MMP-14 6767 A and 6727 G allele carriers have a lower risk for HCC when compared with their corresponding wild-type genotypes. The 6767 G/A polymorphism cause asparagine amino acid in the protein residue to be substituted for aspartic acid. This might alter the original function of MMP-14 protein. The low risk of having HCC might be due to inactivation or reduced expression of MMP-14 which could be attributed to the presence of A allele. Thus the cell behaves in a strange way [15].

MMP14-6727 C/G polymorphism might be involved in disturbed stability of MMP-14 protein or change the translational rate or MMP14 mRNA. It is a synonymous SNP that is located in exon 5. However, its association with cancer has not been reported [37].

5. CONCLUSION

In conclusion, genotyping of related SNPs might provide an easy and important method to foresee the risk and the prognosis of cancer. MMP-1 -1607 2G allele carriers would increase the risk of HCC only in HCV infected patients. People with MMP-9-1562 T, 6727 C and 6767 G allele carriers and homozygote genotype might help to prediction of susceptibility and development of HCC.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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