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Matrix Metalloproteinase-1 gene polymorphism is associated with the prognosis of Hepatocellular Carcinoma in Egyptian population

KEYWORDS: MMP-1, gene polymorphism, hepatocellular carcinoma, prognosis, hepatitis virus C.

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ABSTRACT *This study aimed to examine the relationship between the gene polymorphism of MMP-1 and the prognosis of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) in Egyptian population. Methods: The study enrolled 180 Egyptian individuals classified into 3 groups Group I: consists of 60 apparently healthy individuals served as control Group II: consists of 60 individuals diagnosed with HCV Group III: consists of 60 individuals diagnosed with HCC. Gene polymorphism of MMP-1 -1607 1G/2G using restriction fragment length polymorphism (RFLP) for amplified genomic DNA was analyzed. Results: In HCC prognosis, MMP-1 2G carriers had higher probability of developing HCC when compared to healthy individuals. Conclusion: MMP-1 2G allele may be a cooperative risk factor for poor prognosis in HCC patients, suggesting that further studies with larger sample size should be investigated to ensure that this gene polymorphism might be a potential marker for predicting the prognosis of HCC patients in Egyptian patients.*

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INTRODUCTION

Hepatocellular carcinoma (HCC) is ranked to be the most common cancer in many countries. Recently, HCC was reported to be the fifth most common cancer in males, the eighth common cancer in females and about 560 000 cases are discovered per year, more than 80% of which occur in the developing countries (Abdel-Hamid NM et al., 2009). Egypt is known for being the country in the world where the rate of HCV is higher, about 24% of the people are estimated to carry HCV and the more than 50% of blood donors have anti-HCV in some towns. Chronic infection with hepatitis C virus (HCV) is considered one of the major causes of end-stage liver disease including cirrhosis and hepatocellular carcinoma (Sene Waly Raphael et al., 2012) ;(Nelson PK et al., 2011).

Recent studies have highlighted the role of the ECM and shown the importance of deregulated ECM dynamics in molecular etiology of cancer development (Frantz C et al., 2010). Degradation of extracellular matrix is required for tumor cell migration and dissemination, a process that is facilitated by a family of neutral proteolytic enzymes known as the matrix

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metalloproteinases (MMPs) (A Hettiaratchi et al., 2007).

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that are capable of degrading various components of the extracellular matrix. They are involved in all stages of cancer progression, not only in the process of tumour invasion and metastasis, but also in as proliferation, adhesion, migration, differentiation, angiogenesis and apoptosis (Patricia González-Arriaga et al., 2012).The human MMP family currently consists of 28 members of homologous zinc-dependent endopeptidases, secreted as pro- enzymes and activated after the removal of their N-terminal domain (A. Altadill et al., 2009); (Necati TASKIN et al., 2011). Matrix metalloproteinases (MMPs) are implicated in cancer development and progression and are associated with prognosis. Single-nucleotide polymorphisms (SNPs) of MMPs, most frequently located in the promoter region of the genes, have been shown to influence cancer susceptibility and/or progression (Alexandra MJ Langers et al., 2011).

The interstitial collagenase-1 (MMP-1) is one of the principal proteinases that possesses proteolytic activity against interstitial collagens, the most abundant classes of ECM proteins in fibrotic livers (Kinya Okamoto et al., 2005). The gene is part of a cluster of MMP genes which localize to chromosome 11q22.3 with 11 exons and10 introns (M. C. Kazimi et al., 2010). MMP-l promoter gene polymorphism is the insertion/deletion of a guanine (G) at position -1,607, and has two alleles, one with a single guanine (1G) and the other with two (2G) (Okamoto K et al., 2010). The insertion of a second G nucleotide at position -1607 of MMP1 (-1607insG, rs1799750) generates a new 5'-GGA'3 sequence that corresponds to a recognition sequence for members of Ets family of transcriptional factors (T. Vlaykova et al., 2011). Many studies have proved that cells containing 2G polymorphism (1G/2G or 2G/2G), which created Ets binding sites, were found to be more transcriptionally active than cells with 1G/1G genotype (Bo Pengy et al., 2010).

MATERIALS AND METHODS:

Site of the study

The study was carried out in Biochemistry and Internal Medicine Departments - Faculty of medicine, Zagazig university.

Subjects

The present study was carried out on 180 Egyptian individuals (71 females and 109 males) with age ranged from (39 to 61 years). They were divided into the following groups: Group I: consists of 60 apparently healthy individuals served as control (39 males and 21 females) with ages ranged from 39 to 61 with a mean value ± SD of (50.32 ± 6.84) Group II: consists of 60 individuals diagnosed with HCV (36 males and 24 females with ages ranged from 43 to 61 with a mean value ± SD of 50.5 ± 5.45) Group III: consists of 60 individuals diagnosed with HCC (34 males and 26 females) with ages ranged from 44 to 59 with a mean value ± SD of (52 ± 4.48).

All individuals were subjected to the following:-

- Measurement of AFP level by the third generation ELISA using kits from the Equipar (Saronno,Italy).
- Measurement of liver enzymes (ALT & AST) by Bayer Opera Chemistry System (Diagnostic Division Tarrytown, NY USA).
- Determination of MMP-1 gene polymorphism by PCR amplification followed by restriction Fragment length polymorphism (RFLP) and gel electrophoresis.

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DNA extraction

Genomic DNA was isolated from 3 ml venous blood sample withdrawn on EDTA using genomic DNA purification kit (Fermentas) according to the manufacturer's instructions.

Genotype analysis

Gene polymorphisms were detected through PCR amplification followed by digestion using restriction endonuclease enzymes for RFLP analysis. MMP1 gene 1G/2G polymorphism was genotyped using the forward primer (5'-TCGTGAGAATGTCTTCCCATT-3'); and the reverse primer (5'-TCTTGGATTGATTTGAGATAAGTGAA ATC -3'), according to previous reports (Dunleavey L. et al., 2000).

PCR reaction for both polymorphisms

PCR reaction was performed in a final volume of 50µl that contained: 2X PCR Mix: 25 µl , Primer mix (2.5 µM or 1/40 0f dilution 100 µM stock): 1µl for each primer, Genomic DNA: 5 µl and Deionized water: 18 µl. The amplification was carried out using DNA thermal cycler 480, PERKIN ELMER (Norwalk, CT 06856, USA), Serial No. P 16462.PCR conditions for MMP 1 polymorphism were; 1 min cycle for initial denaturation at 95 ˚C; 35 cycles at 95 ˚C for 1 min for denaturation, 55 ˚C for 30 sec for annealing and 72 ˚C for 30 sec for extension, followed by 1 cycle at 72 ˚C for 5 minutes for final extension (Dunleavey L. et al., 2000).

Restriction enzyme digestion

PCR products were digested with restriction endonucleases (Fast Digest, Thermo Scientific) and subjected to electrophoresis on a 2% agarose gel and the bands were visualized by ethidium bromide staining under U/V light.

For MMP-1 -1607 (1G/2G)

Digestion of the PCR fragments with Xmn I produced 117, 89 and 28 bp for and 117 bp for *2G allele* [15]. (Figure 1).

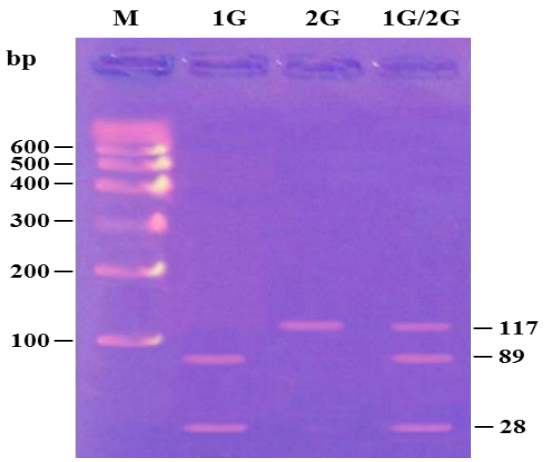

Figure 1: shows a 2% agarose gel picture, stained with ethidium bromide, products digested with Xmn I . M lane: 100 bp - 1kb DNA ladder; lan 1: 1G homozygote (89 bp + 28 bp); lan 2: 2G homozygote (117 bp); lan 3: 1G/2G heterozygote (117 bp + 89 bp + 28 bp).

Statistical methods

All statistical analysis was performed using the statistical package for social science (SPSS) version 11 (Chicago, IL, USA) [16]. Data were statistically described in terms of mean± standard deviation (±SD), range, or frequencies (number of cases) and percentages when appropriate. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated for all studied polymorphism haplotypes and alleles between cases and controls. P values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of participants

This study was carried out on 180 individuals classified into 3 groups: Group I: Control group (n=60): Including 39 (60.6%) males and 21 (39.4%) females with ages ranged from 39 to

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61 with a mean value ± SD of 50.32 ± 6.84). Group II: HCV group (n=60): Patients positive for serum HCV Including 36 (60%) males and 24 (40%) females with ages ranged from 43 to 61 with a mean value ± SD of 50.5 ± 5.45). Group III: HCC group (n=60): patients diagnosed with HCC including 34 (56.7%) males and 26 (43.3%) females with ages ranged from 44 to 59 with a mean value ± SD of 52 ± 4.48). Table 1 shows the laboratory data of the individuals in the three groups.

Table 1: The association between serum AFP, ALT and AST level in different studied groups

	Controls (n =60)		HCV (n=60)		HCC (n=60)		
Parameters	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	*** P-value**
AFP (ng/ml)	1.914	0.828	10.964	2.277	485.978	636.950	<0.001
ALT (U/L)	24.816	3.702	40.666	30.836	238.601	256.150	<0.001
AST (U/L)	26.683	4.416	46.883	41.884	203.249	220.283	<0.001

** p>0.05 is considered non-significant; p<0.05 is considered significant*

Genotypes of MMP-1 gene.

2G/2G genotype: a higher representation of the 2G/2G genotype was found in HCC group as compared to control group & in HCV group as compared to control group. Statistical significance was observed between Control & HCC (P=0.029; OR = 2.25; CI = 1.0838 - 4.6710), a higher representation of the 2G/2G genotype was found in the HCC group when compared to the HCV group (Tables 2).

1G/2G genotype: 1G/2G genotype was underrepresented when comparing control & HCV group and control & HCC. A Slightly higher representation of the 1G/2G genotype was found between HCV group as compared to HCC group but didn't reach statistical significance (Tables 2).

1G/1G genotype: There was no significant difference in representation of 1G genotype between different studied groups (Tables 2).

There was a statistically significant association between 2G/2G genotype in HCC group as compared to the control (P value <0.05).

Table 2: Genotypes distributions of MMP-1 gene polymorphisms among control and different patient groups.

Polymorphism	Cases n= (%)	Control n=60 (%)	OR	95 % CI	*P-value
MMP-1 gene polymorphism					
HCV (n=60)					
1G	9 (15)	10 (16.7)	1 (reference)		
1G/2G	21 (35)	26 (43.3)	0.7041	0.3372 - 1.4704	0.3505
2G	30 (50)	24 (40)	1.5	0.7279 - 3.0912	0.2718
HCC (n=60)					
1G	7 (11.7)	10 (16.7)	1 (reference)		
1G/2G	17 (28.3)	26 (43.3)	0.5170	0.2420 - 1.1044	0.0884
2G	36 (60)	24 (40)	2.25	1.0838 - 4.6710	**0.0296**

** p>0.05 is considered non-significant; p<0.05 is considered significant*

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1G allele: a higher representation of 1G allele was present in the Control group when compared with both HCC and HCV groups but didn't reach statistical significance (Tables 3).

2G allele: a statistically higher representation of 2G allele was present in the HCC group when compared with both HCV and Control groups (P=0.039) (Tables 3).

Risk Assessment: association of 2G genotype & HCC showed that 2G/2G individuals have 2.25 times higher probability of developing HCC when compared to healthy individuals.

There is a statistically significant allelic association between HCC group & Control group (P=0.039;OR=1.78) 2G/2G individuals are in risk of developing HCC; individuals without 2G allele may be protected from the disease.

Table 3: Allele frequency of MMP-1 gene polymorphisms among control and different patient groups

Polymorphism	Cases n= (%)	Control n=60 (%)	OR	P-value
MMP-1 Allele frequencies				
HCV (n=60)				
1G	39 (32.5)	46 (38.33)	0.77	0.345
2G	81 (67.5)	74 (61.67)	1.29	
HCC (n=60)				
1G	31 (25.83)	46 (38.33)	0.5603	**0.039**
2G	89 (74.16)	74 (61.67)	1.78	

** p>0.05 is considered non-significant; p<0.05 is considered significant*

DISCUSSION:

This study evaluated the effect of -1607 1G/2G polymorphisms in the promoter region of MMP-1 on the Prognosis of Hepatocellular Carcinoma in Egyptian population.

Many studies have proved that cells containing 2G polymorphism (1G/2G or 2G/2G), which created Ets binding sites, were found to be more transcriptionally active than cells with 1G/1G genotype (Bo Pengy et al., 2010). Study results showed that 2G/2G individuals have 2.25 times higher probability of developing HCC when compared to healthy individuals with a statistically significant difference (P = 0.0296, CI= 1.0838 - 4.6710). These findings are in accordance with Okamoto K et al .,2005 who reported that In MMP-1 genotypes, the 2G homozygotes were significantly more in cirrhotic group than in chronic hepatitis group. Also, B.K. Jang et al (B.K. Jang et al., 2009) reported that SNPs of the MMP1 gene contribute to genetic susceptibility to HCC in Korean population. In a recent study Mohy Eldin et al., 2013 reported that MMP-1 is overexpressed in a large proportion of Egyptian patients with HCC and the high expression level of protein correlated with the disease progression and poor clinical outcome in HCC. Furthermore, MMP-1 high expression proved to be a risk factor for tumor recurrence and independent molecular marker of prognosis in HCC and may become a novel target in the strategies for the prediction of tumor progression and prognosis of this disease. In other carcinomas, Bradbury et al., 2009 reported

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that 1G/2G and 2G/2G individuals are associated with increased esophageal adenocarcinoma risk (In a Caucasian population, 313 cases & 455 controls).Also Kouhkan et al., 2008 and Woo et al., 2007 reported that 2G/2G individuals are associated with increased Colorectal cancer risk (In an Iranian population, Korean Population - 150,185 cases & 100,304 controls, respectively).

There are also other studies that are not in agreement with our findings, Zhai Y et al., 2007 who reported that there is no association between MMP1 -1607 polymorphism and HCC progression in Chinese patients (434 cases and 480 controls). Also, S.Nalbantoglu et al (11) reported that although 2G/2G genotype was associated with portal vein invasion ($P<0.02$), There was no statistically significant difference in the genotype distributions ($P = 0.38$) or allele frequencies ($P = 0.236$) of MMP1 -1607 1G/2G between cases and controls in Turkish population.

There are number of factors which affect Controversial results on polymorphism- disease association studies which also will explain why this study results are not in agreement with previous mentioned studies. Ethnicities of the races; People of different populations have different genetic backgrounds and may be exposed to different environment factors, so the same polymorphism may play different roles in different populations. Also heterogenetic nature of cancer diseases and Different MMP regulation mechanisms and microenvironment in different tissues may explain why the same polymorphism plays different roles in different types of cancer.

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