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Matrix metalloproteinase-9 gene polymorphism in hepatocellular carcinoma patients with hepatitis B and C viruses

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ABSTRACT. Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide. In Egypt, the incidence of HCC has doubled over the last decade. Matrix metalloproteinase-9 (MMP-9) plays a key role in cancer invasion and metastasis by degrading the extracellular matrix and basement membrane barriers. A cytosine (C)/thymidine (T) single nucleotide polymorphism at position -1562 in the MMP-9 promoter is reported to influence the expression of the MMP-9 gene. The association between MMP-9 gene polymorphisms and HCC

patients with hepatitis C and B viruses (HCV and HBV) was examined in 91 patients with HCC and viral hepatitis (55 HCV and 36 HBV). The results were compared with those of 42 HCC patients without viral hepatitis and 60 healthy individuals with no liver infection. Polymorphisms of the MMP-9 gene were investigated by polymerase chain reaction amplification followed by restriction fragment length polymorphism analysis. The serum MMP-9 level was quantitatively determined using a human MMP-9 enzyme-linked immunosorbent assay, which showed that homozygosity of the MMP-9 promoter (TT) was more frequent in patients with HCC and chronic HCV or HBV infection when compared with the control group (49.1, 52.8, and 35.7%, respectively). In addition, we observed significant elevation of serum MMP-9 levels in all HCC groups compared to controls. It was concluded that patients with the MMP-9 TT genotype are at risk of developing HCC and HBV or HCV. People with significantly elevated serum levels of MMP-9 are at risk of developing HCC.

Key words: MMP-9 gene polymorphism; Viral hepatitis; Hepatocellular carcinoma

INTRODUCTION

Egypt is an endemic area of hepatitis C virus (HCV). Globally, 1 in 50 people are infected with HCV, whereas approximately 1 in 7 of Egypt's 83 million people tested positive for antibodies against HCV. However, nearly 1 in 10 people carry its viral RNA and are therefore chronically infected (Yahia, 2011).

Hepatocellular carcinoma (HCC) is the fifth most common tumor and the third most common cause of cancer-related deaths worldwide (Okamoto et al., 2010). Carcinogenesis of HCC is a multistep and complex process, and it is known that multiple risk factors, including chronic hepatitis B virus (HBV) or HCV infection, cirrhosis, carcinogen exposure, and a variety of single nucleotide polymorphisms, contribute to hepatocarcinogenesis (Weng et al., 2010). The HCC incidence has increased sharply in recent decades, which can be partially attributed to the increase in chronic HCV infection (Bosch et al., 2005). In Egypt, the growing incidence of HCC has nearly doubled over the last decade (National Cancer Registry of Egypt, 2010). In addition, Egypt has the highest prevalence of HCV in the world, at 6-28% (Egyptian Ministry of Health, 2007), and the prevalence of HCV serological markers in patients with HCC is nearly 80% (Lehman and Wilson, 2009).

Matrix metalloproteinase (MMPs) are a family of zinc-dependent extracellular endopeptidase enzymes that play a vital role in the proteolysis of structural and signaling components of the extracellular matrix (ECM) and influence cell differentiation, migration, invasion, and proliferations of cells (Weng and Yen, 2010). Since HCC tumors develop in fibrotic livers, MMP activity is particularly necessary for growth, invasion and metastasis. Indeed, the levels of MMPs are frequently increased in HCC tumor tissues, and some previous studies have suggested that intratumoral MMP overexpression is closely correlated with the potential for HCC invasion and metastasis (Yamamoto et al., 1997).

MMP-9, a known gelatinase B or type IV collagenase of 92 kDa is the major MMP produced by human macrophages. Its substrates include basement membrane collagen types IV and V, different types of gelatin, fibronectin, and elastin (Senior et al., 1991). MMP-9 is a proteolytic enzyme necessary for extravasation, migration and tissue remodeling during chronic inflammation (Watanabe et al., 1993).

The human MMP-9 gene is located on chromosome 20q11.2-20q13.1 and contains 13 exons and 12 introns (O-Charoenrat et al., 2001). A single-nucleotide polymorphism C/T transition at position -1562 in the promoter region (rs3918242) has a functional effect on gene expression that prevents binding of a nuclear protein in the promoter region of MMP-9 in macrophages. Cells with the C/C genotype show low transcriptional activity, while those with C/T and T/T genotypes show MMP-9 transcriptional activity (Folgueras et al., 2004). Harboring of the T allele of the -1562 C/T MMP-9 gene polymorphism is related to an increased risk of specific cancers and tumorigenesis (Sugimoto et al., 2006). MMP-9 overexpression was found to be associated with capsular invasion, vascular invasion, tumor stage, and intrahepatic metastasis in HCC (Chen et al., 2009).

Elevated serum alpha fetoprotein (AFP) is known to be associated with HCC. Because AFP is also elevated in benign conditions of acute and chronic hepatitis, the use of AFP lacks specificity for HCC diagnosis (Johnson, 2001). Des-gamma-carboxyprothrombin (DCP) is an abnormal prothrombin that lacks carboxylation of specific amino-terminal glutamic acid residues. The DCP level has recently been reported to be closely correlated with tumor progression and prognosis (Nakamura et al., 2006). In this study, we investigated the association between MMP-9 gene polymorphisms and HCC in patients with HCV or HBV.

MATERIAL AND METHODS

Patients

The study included 193 cases: 133 patients attending the Oncology Center, Mansoura University, and suffering from HCC, 91 patients with HCC and viral hepatitis (55 HCV and 36 HBV), and 42 patients with HCC without viral hepatitis. The control population comprised 60 healthy individuals with no liver infection. Patients were subjected to a full history and thorough physical examination. General physical examination included examination of metabolic, endocrine, cardiovascular, respiratory, gastro-intestinal, and neurological systems to exclude the presence of abnormalities. The liver was examined in all patients by local physical examination to detect possible abnormalities. HCC was diagnosed by ultrasonography and computed tomography as well as by liver histopathological examinations. Other features, including gender, age, tumor size, liver function tests, and the serum tumor marker AFP level, were determined. Patients with HCC and viral hepatitis infection included in the study were positive for serum HCV or HBV RNA. Exclusion criteria included diabetes mellitus, chronic renal failure, coronary artery disease, end-stage liver disease, and positive serum antinuclear antibody. Neither patients nor controls had a history of other malignancy. All subjects were informed of the purpose of the study and informed consent was obtained. The study was approved by the Ethics Committee of Mansoura University.

Biochemical investigation

All subjects were instructed to fast for at least 12 h. One-milliliter blood samples were collected and stored as ethylenediamine tetraacetic acid (EDTA) anti-coagulated blood sample at -30°C for DNA extraction. An additional 5 mL blood was allowed to clot for 15 min and centrifuged for 10 min for serum separation to determine serum MMP-9, AFP, and DCP levels.

DNA extraction and investigation of th*e* -1562 C/T polymorphism in the promoter of the MMP-9 gene

Genomic DNA was extracted from peripheral blood using a Gentra genomic DNA purification kit (Qiagen, Hilden, Germany). The region containing the restriction fragment length polymorphism (RFLP) within the MMP-9 gene was amplified using Taq DNA polymerase. A set of primers was designed to amplify a 435-bp fragment that included -1562 C/T in the promoter of the MMP-9 gene polymorphism (Tu et al., 2007). The forward primer used was 5'-GCCTGGCACATAGTAGGCCC-3' and the reverse primer used was 5'-CTTCCTAGCCAGCCGGCATC-3'.

Each polymerase chain reaction (PCR) cycle used 300 ng DNA, 200 mM dNTPs, 500 nM primer, and 2.5 U *Taq* DNA polymerase (Amplitaq Gold, Perkin-Elmer, Waltham, MA, USA). DNA was initially denatured for 2 min at 95°C, and then PCR amplification was performed via 30 cycles using the following temperature program: denaturation at 95°C for 45 s, annealing at 67°C for 45 s, and extension at 72°C for 45 s. PCR amplification was completed by a final extension at 72°C for 7 min. The amplification yielded a 435-bp product.

After cleavage with 5 U *Sph*I (Cat. No. 15413-016; Life Technologies, Invitrogen, Carlsbad, CA, USA) for 3 h at 37°C, the DNA fragments were detected on a 3% agarose gel stained with ethidium bromide and visualized under ultraviolet light. Allele T produced 2 bands of 247 and 188 bp, whereas the C allele remained intact. Agarose gel electrophoresis revealed 3 patterns: normal genotype CC with 435-bp fragments, heterozygous mutated genotype CT with 435-, 247-, and 188-bp fragments, and homozygous mutated genotype CC with 247- and 188-bp fragments (Okamoto et al., 2010).

Estimation of serum human MMP-9 level

The serum MMP-9 level was quantitatively determined by using a solid phase enzyme-linked immunosorbent assay (ELISA) with a Human MMP-9 ELISA kit according to manufacturer instructions (RayoBio, Norcross, GA, USA). The absorbance of each sample was read on an ELISA plate reader (Tecan, Maennedorf, Switzerland) at 450 nm (Borkakoti, 1998).

Estimation of serum AFP level

The serum of AFP level was determined using an ELISA Kit (Quantikine Human AFP Immunoassay Kit; R&D Systems, Minneapolis, MN, USA). The assay was performed according to manufacturer instructions. The absorbance of each sample was read on an ELISA plate reader at 450 nm with a correction wavelength of 570 nm (Canick et al., 2003).

Estimation of serum DCP

The DCP level was determined using ELISA (Eiest PIVKAII; Eisai Co., Tokyo, Japan) according to manufacturer instructions. The absorbance of each sample was read on an ELISA plate reader at a wavelength of 450 nm. The detection limit was 10 mAU/mL. The cut-off value was determined to be 40 mAU/mL for the differentiation of HCC and non-malignant liver disease based on previous studies (Mita et al., 1998).

Statistical analysis

Statistical analysis was concluded using the Excel program and SPSS version 10. Means ± standard deviation (SD), and the frequency and proportion for qualitative data were determined. Statistical significance was determined between groups. For quantitative analysis, the Student *t*-test was used to compare 2 groups; one-way analysis of variance was used to compare more than 2 groups. The chi-square test was used to compare qualitative data. $P < 0.05$ was considered to be significant at a confidence interval of 95%.

RESULTS

Descriptive and biochemical data in the control group and HCC patients are shown in Table 1. AFP, MMP-9, and DCP are significantly elevated in all HCC group compared to the control group. MMP-9 was significantly elevated in HCC patients with HBV when compared to that in HCC patients without viral hepatitis.

Table 1. Descriptive and biochemical data in the control group and HCC patients.

	Control group (N = 60)	HCC group		
		HCC with HCV (N = 55)	HCC with HBV (N = 36)	HCC without HCV or HBV (N = 42)
Age (years)	59.02 ± 6.82	60.73 ± 5.84	58.46 ± 4.51	66.64 ± 4.66
Sex [N (%)]				
Male	22 (48.9%)	24 (64.9%)	16 (66.7%)	14 (50%)
Female	23 (51.1%)	13 (35.1%)	8 (33.3%)	14 (50%)
Tumor size (cm)	0	3.08 ± 0. 81	2.94 ± 0.79	2.98 ± 0.73
AFP (μg/mL)	10.45 ± 5.7	1828 ± 1426.4[a]	2007.67 ± 1189.21[a]	1417.6 ± 1468.7[a]
MMP-9 (ng/mL)	38.51 ± 16.45	255.51 ± 129.51[a]	321 ± 126.23[a,b]	233.18 ± 133.43[a]
DCP (mAU/mL)	25.73 ± 10.14	3260.92 ± 3421.9[a]	1933.88 ± 2415.9[a]	2183.18 ± 2961.85[a]

Data are reported as means ± SD, unless otherwise indicated. [a]Significant difference from control group. [b]Significant difference from HCC without virus group.

PCR determination of polymorphism -1562 C/T in the promoter of MMP-9 gene (Figure 1) revealed a significant increase in the TT genotype frequency in patients with HCC with viral hepatitis compared to that in normal individuals ($P < 0.05$). In addition, there was a significant increase in T allele frequency in HCC patients with viral hepatitis compared to that in normal individuals ($P < 0.05$). However, there was no significant difference in the genotype distribution between HCC patients without viral hepatitis and normal individuals ($P > 0.05$) (Table 2).

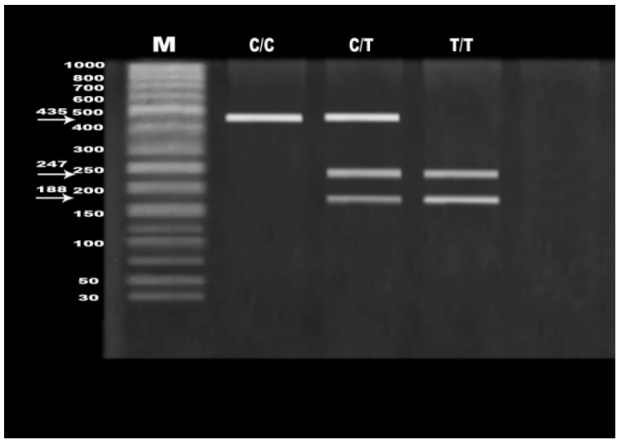

Figure 1. Ethidium bromide-stained 2% agarose gel showing enzymatic digestion of the -1562 C/T polymorphism of the MMP-9 gene for different groups studied. *Lane M* = 50-bp DNA size marker in base pairs labeled from the bottom up (50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800).

Table 2. Genotype distribution and allele frequency of the MMP-9 -1562 polymorphism in the control group and HCC patients.

	Control group (N = 60)	HCC group		
		HCC with HCV (N = 55)	HCC with HBV (N = 36)	HCC (Without virus) (N = 42)
TT genotype	16 (26.7%)	27 (49.1%)[a]	19 (52.8%)[a]	15 (35.7%)
TC genotype	23 (38.3%)	16 (29.1%)	9 (25.0%)	13 (31%)
CC genotype	21 (35%)	12 (21.8%)	8 (22.2%)	14 (33.3%)
T allele	55 (45.8%)	70 (63.6%)[a,b]	47 (65.3%)[a,b]	43 (51.2%)
C allele	65 (54.2%)	40 (36.4%)[a,b]	25 (34.7%)[a,b]	41 (48.8%)

[a]Significant difference from control group. [b]Significant difference from HCC only group.

Table 3 shows the odds ratio (OR) for the TT genotype of the MMP-9 gene between the control group and the HCC group. The TT genotype of the MMP-9 gene showed a significant difference between patients with HCC and HCV or HBV and the control group (OR = 2.65, 95% confidence interval (CI) = 1.14-6.24 and OR = 3.07, 95%CI = 1.2-8.7, respectively). In addition, a significant difference was observed in the TT genotype of the MMP-9 gene between patients with HCC and the control group (OR = 2.33, 95%CI = 1.14-4.8).

Table 3. Odds ratios for MMP-9 TT genotypes between the control group and HCC patient groups.

	Control group (N = 60)	HCC group			
		HCC with HCV (N = 55)	HCC with HBV (N = 36)	HCC without HCV or HBV (N = 42)	Total HCC group (N = 133)
Positive	16	27	19	15	61
Negative	44	28	17	27	72
P value	Reference	0.01	0.01	0.3	0.01
OR		2.65	3.07	1.53	2.33
95%CI		1.14-6.24	1.2-8.7	0.6-3.9	1.14-4.8

OR = odds ratio; 95%CI = 95% confidence interval.

Table 4 presents biochemical parameters in different genotypes and alleles of MMP-9 in all groups. There were no significant differences in the levels of AFP, MMP-9, and DCP between different genotypes and alleles of the MMP-9 gene polymorphism.

Table 4. Biochemical parameters in different genotypes and alleles of the MMP-9 polymorphism in all groups.

Parameters	MMP-9 genotype			P value	MMP-9 alleles		P value
	TT (N = 77)	TC (N = 61)	CC (N = 55)		T (215)	C (171)	
AFP (μg/mL)	1177.88 ± 1570.2	1241.25 ± 1435.7	1102.85 ± 1249.5	0.88	1186 ± 1300.56	1172 ± 1511.36	0.461
MMP-9 (ng/mL)	183.67 ± 157.4	199.84 ± 166.9	185.52 ± 142.8	0.87	185.33 ± 126.57	190.43 ± 143.06	0.694
DCP (mAU/mL)	1935 ± 2552.1	1345 ± 2224.3	1665 ± 2290.1	0.32	1623 ± 2788.62	1494 ± 2433.55	0.207

Data are reported as means ± SD.

DISCUSSION

HCC is a frequent malignancy worldwide. HCV and HBV are considered major etiological factors associated with the development of HCC, particularly because of their induction during chronic inflammation (Budhu and Wang, 2006). MMP levels are elevated in different types of human cancers and play a crucial role in cancer development. The proposed role of MMP in tumor invasion is based mainly on the observed high-level expression of distinct MMPs in malignant tumors (Przybylowska et al., 2006).

In the current study, we observed an increased frequency of MMP-9 in the TT genotype and the T allele in HCC patients with viral hepatitis when compared to the control group ($P < 0.05$), but there was no significant difference in the MMP-9 TT genotype and the T allele between HCC patients without viral hepatitis and the control group ($P > 0.05$).

Okamoto et al. (2010) observed a positive relationship between MMP-9 T carriers and poor HCC differentiation, but could not demonstrate a significant relationship between MMP-9 -1562 C/T genotypes with either the development or prognosis of HCC. MMP-9 mRNA levels in HCC tissue are frequently up-regulated through the activation of the PI3K, AKT, and nuclear factor-κB pathways and are correlate to HCC capsular infiltration (Chen et al., 2009).

It is not yet clear why poorer HCC histological grades, which appears to correlate with the MMP-9 -1562 T allele, are not correlated with overall survival. However, this discrepancy is likely to result from different balances of MMP-9 multiple functions. For example, MMP-9 cleaves plasminogen to generate angiostatin, one of the most potent inhibitors of angiogenesis (Vairaktaris et al., 2008). 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 (Okamoto et al., 2010).

The association between MMP-9 -1562C/T polymorphisms and the development of malignant phenotypes has been investigated for a variety of cancers. However, the implications of MMP-9 -1562C/T polymorphisms in carcinogenesis and progression vary greatly in different types of human malignancy (Sfar et al., 2007). Wu et al. (2008) found that MMP-9 C-1562T polymorphisms had no influence on the risk of recurrent HCC after liver transplantation.

The current study revealed significant increase in the serum level of MMP-9 in all HCC groups when compared with the control group, and which was also, significantly increased in

HCC patients with HBV when compared with HCC cases due to non-viral factors. MMP-9 plays an important role in the distant metastatic potential of cancer cells, because of its type IV collagenolytic activity, which is known to be associated with disruption of the basement membrane (Jones and Walker, 1997). Angiogenesis is a very important step in cancer progression because it promotes both primary tumor growth and metastasis dissemination. MMP-9 activity is required to produce a normal pattern of vascularization; therefore, an increased level of MMP-9 is associated with highly vascularized and rapidly growing tumors (Chantrain et al., 2004).

Several studies have also reported that MMP-9 expression be associated with the progression and invasion of brain cancer, glioma and breast cancer (Scorilas et al., 2001). Overexpression of MMP-9 is associated with capsular infiltration of HCC and the growth of small HCC (Sakamoto et al., 2000). Chung et al. (2004) observed enhanced expression of MMP-9 and carcinogenesis by HBV infection into liver cells. In addition, elevated plasma levels of MMP-9 have been observed in patients with HCC, particularly in those with macroscopic portal vein invasion.

We found no significant difference in the serum level of MMP-9 in different genotypes and alleles of the MMP-9 polymorphism. Przybylowska et al. (2006) did not find the influence of C/T polymorphism in the MMP-9 gene on MMP-9 expression in breast cancer. This may be because repressor protein binding to the sequence with the C allele is not expressed in breast cancer cells.

MMP-9 expression levels can be influenced by genetic variation in MMP-9 gene promoters. The sequences in the promoter region of the MMP-9 gene containing the C/T (-1562 bp) polymorphic site constitute an important regulatory element that appears to be a binding site for a transcription repressor protein. Therefore, C/T substitution at the polymorphic site abolishes the DNA-protein interaction, resulting in a higher activity of the T-allelic promoter (Zhang et al., 1999).

Okamato et al. (2005) found that the prevalence of the MMP-9 C allele was significantly greater in liver cirrhosis patients than in chronic hepatitis patients, suggesting that the MMP-9 C allele and C homozygote may contribute to the progression of liver fibrosis. Therefore, C homozygotes showing low transcriptional activity have lower production of MMP-9 in the fibrotic liver, resulting in rapid progression of liver fibrosis due to decreased degradation of the ECM.

DCP and AFP were significantly elevated in all HCC groups because of viral hepatitis or other causes. Several studies have found that an elevated serum DCP level is correlated with the presence of vascular invasion or intrahepatic metastases of HCC (Hakamada et al., 2008).

DCP, an aberrant prothrombin produced by HCC cells, was found to be associated with the biological malignant potential of HCC. DCP stimulates HCC growth and promotes HCC metastasis by increasing MMP-2 and MMP-9 activity by activation the ERK1/2 MAPK signaling pathway (Yue et al., 2011).

CONCLUSIONS

Individuals with the MMP-9 TT genotype are at risk of developing HCC with chronic HCV or HBV. In addition to AFP and DCP, MMP-9 genotyping is a possible marker for early prediction and prognosis of HCC. Further studies in larger population samples are recommended to explain and confirm these findings.

REFERENCES

Borkakoti N (1998). Matrix metalloproteases: variations on a theme. *Prog. Biophys. Mol. Biol.* 70: 73-94.

Bosch FX, Ribes J, Cleries R and Díaz M (2005). Epidemiology of hepatocellular carcinoma. *Clin. Liver Dis.* 9: 191-211.

Budhu A and Wang XW (2006). The role of cytokines in hepatocellular carcinoma. *J. Leukoc. Biol.* 80: 1197-1213.

Canick JA, Kellner LH and Bombard AT (2003). Prenatal screening for open neural tube defects. *Clin. Lab. Med.* 23: 385-94.

Chantrain CF, Shimada H, Jodele S, Groshen S, et al. (2004). Stromal matrix metalloproteinase-9 regulates the vascular architecture in neuroblastoma by promoting pericyte recruitment. *Cancer Res.* 64: 1675-1686.

Chen JS, Wang Q, Fu XH, Huang XH, et al. (2009). Involvement of PI3K/PTEN/AKT/mTOR pathway in invasion and metastasis in hepatocellular carcinoma: Association with MMP-9. *Hepatol. Res.* 39: 177-186.

Chung TW, Kim JR, Suh JI, Lee YC, et al. (2004). Correlation between plasma levels of matrix metalloproteinase (MMP)-9 /MMP-2 ratio and alpha-fetoproteins in chronic hepatitis carrying hepatitis B virus. *J. Gastroenterol. Hepatol.* 19: 565-571.

Egyptian Ministry of Health (2007). Egyptian Ministry of Health Annual Report. Available at [http://www.mohp.gov.eg/Main.asp]. Accessed July 6, 2010.

Folgueras AR, Pendás AM, Sánchez LM and López-Otin C (2004). Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *Int. J. Dev. Biol.* 48: 411-424.

Hakamada K, Kimura N, Miura T, Morohashi H, et al. (2008). Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma. *World J. Gastroenterol.* 14: 1370-1377.

Johnson PJ (2001). The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin. Liver Dis.* 5: 145-159.

Jones JL and Walker RA (1997). Control of matrix metalloproteinase activity in cancer. *J. Pathol.* 183: 377-379.

Lehman EM and Wilson ML (2009). Epidemiology of hepatitis viruses among hepatocellular carcinoma cases and healthy people in Egypt: a systematic review and meta-analysis. *Int. J. Cancer* 124: 690-697.

Mita Y, Aoyagi Y, Yanagi M, Suda T, et al. (1998). The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer* 82: 1643-1648.

Nakamura S, Nouso K, Sakaguchi K, Ito YM, et al. (2006). Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am. J. Gastroenterol.* 101: 2038-2043.

National Cancer Registry of Egypt (2010). Magnitude of Hepatocellular Carcinoma in Egypt. Available at [http://www.nci.edu.eg]. Accessed August 6, 2010.

O-Charoenrat P, Rhys-Evans PH and Eccles SA (2001). Expression of matrix metalloproteinases and their inhibitors correlates with invasion and metastasis in squamous cell carcinoma of the head and neck. *Arch. Otolaryngol. Head Neck Surg.* 127: 813-820.

Okamoto K, Mimura K, Murawaki Y and Yuasa I (2005). Association of functional gene polymorphisms of matrix metalloproteinase (MMP)-1, MMP-3 and MMP-9 with the progression of chronic liver disease. *J. Gastroenterol. Hepatol.* 20: 1102-1108.

Okamoto K, Ishida C, Ikebuchi Y, Mandai M, et al. (2010). The genotypes of IL-1 beta and MMP-3 are associated with the prognosis of HCV-related hepatocellular carcinoma. *Intern. Med.* 49: 887-895.

Przybylowska K, Kluczna A, Zadrozny M, Krawczyk T, et al. (2006). Polymorphisms of the promoter regions of matrix metalloproteinases genes MMP-1 and MMP-9 in breast cancer. *Breast Cancer Res. Treat.* 95: 65-72.

Sakamoto Y, Mafune K, Mori M, Shiraishi T, et al. (2000). Overexpression of MMP-9 correlates with growth of small hepatocellular carcinoma. *Int. J. Oncol.* 17: 237-243.

Scorilas A, Karameris A, Arnogiannaki N, Ardavanis A, et al. (2001). Overexpression of matrix-metalloproteinase-9 in human breast cancer: a potential favourable indicator in node-negative patients. *Br. J. Cancer* 84: 1488-1496.

Senior RM, Griffin GL, Fliszar CJ, Shapiro SD, et al. (1991). Human 92- and 72-kilodalton type IV collagenases are elastases. *J. Biol. Chem.* 266: 7870-7875.

Sfar S, Saad H, Mosbah F, Gabbouj S, et al. (2007). TSP1 and MMP9 genetic variants in sporadic prostate cancer. *Cancer Genet. Cytogenet.* 172: 38-44.

Sugimoto M, Yoshida S, Kennedy S, Deguchi M, et al. (2006). Matrix metalloproteinase-1 and -9 promoter polymorphisms and endometrial carcinoma risk in a Japanese population. *J. Soc. Gynecol. Investig.* 13: 523-529.

Tu HF, Wu CH, Kao SY, Liu CJ, et al. (2007). Functional -1562 C-to-T polymorphism in matrix metalloproteinase-9 (MMP-9) promoter is associated with the risk for oral squamous cell carcinoma in younger male areca users. *J. Oral Pathol. Med.* 36: 409-414.

Vairaktaris E, Vassiliou S, Nkenke E, Serefoglou Z, et al. (2008). A metalloproteinase-9 polymorphism which affects its expression is associated with increased risk for oral squamous cell carcinoma. *Eur. J. Surg. Oncol.* 34: 450-455.

Watanabe H, Nakanishi I, Yamashita K, Hayakawa T, et al. (1993). Matrix metalloproteinase-9 (92 kDa gelatinase/type IV collagenase) from U937 monoblastoid cells: correlation with cellular invasion. *J. Cell Sci.* 104: 991-999.

Weng CJ and Yen GC (2010). The *in vitro* and *in vivo* experimental evidences disclose the chemopreventive effects of *Ganoderma lucidum* on cancer invasion and metastasis. *Clin. Exp. Metastasis* 27: 361-369.

Weng CJ, Hsieh YH, Tsai CM, Chu YH, et al. (2010). Relationship of insulin-like growth factors system gene polymorphisms with the susceptibility and pathological development of hepatocellular carcinoma. *Ann. Surg. Oncol.* 17: 1808-1815.

Wu LM, Zhang F, Xie HY, Xu X, et al. (2008). MMP2 promoter polymorphism (C-1306T) and risk of recurrence in patients with hepatocellular carcinoma after transplantation. *Clin. Genet.* 73: 273-278.

Yahia M (2011). Global health: a uniquely Egyptian epidemic. *Nature* 474: S12-S13.

Yamamoto H, Itoh F, Adachi Y, Sakamoto H, et al. (1997). Relation of enhanced secretion of active matrix metalloproteinases with tumor spread in human hepatocellular carcinoma. *Gastroenterology* 112: 1290-1296.

Yue P, Gao ZH, Xue X, Cui SX, et al. (2011). Des-gamma-carboxyl prothrombin induces matrix metalloproteinase activity in hepatocellular carcinoma cells by involving the ERK1/2 MAPK signalling pathway. *Eur. J. Cancer* 47: 1115-1124.

Zhang B, Ye S, Herrmann SM, Eriksson P, et al. (1999). Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 99: 1788-1794.