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MMP-2 gene expression at mRNA level in HBV and HCV infected patients

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Abstract. Matrix metalloproteinases (MMPs) family play a determinative role in the development of liver fibrosis, metastasis, unregulated angiogenesis, and tumor growth. In this study the possible association between the MMP-2 gene expression level and risk of chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections were evaluated in liver transplanted patients. Formalin fixed paraffin embedded (FFPE) liver tissue samples were collected from 225 transplant patients between years 2012 and 2016. The presence of HBV and HCV infections were analyzed in patients studied using molecular and immunologic diagnostic protocols according to the instructions of the manufacturers. Patients were divided to HBV, HCV, and HBV with HCV co-infected groups. A healthy control group was also included. For the quantitative analysis of MMP-2 mRNA gene expression an in-house-SYBR Green Real-Time PCR method was performed. The level of MMP-2 mRNA expression showed a significant increase in all studied viral hepatitis infected patient groups in comparing with healthy controls. The MMP-2 gene expression level increased in HBV infected patients when compared with HCV and HBV with HCV co-infected patients, but not significantly. Results showed a significant increase in MMP-2 expression level in all viral hepatitis single and co-infected liver transplanted patients when compared with the controls and also in HBV infected patients when compered with other viral infected ones, need to confirm in further completed studies.

INTRODUCTION

Hepatocellular carcinoma (HCC) is said to be the 5th most common tumor and also the 3rd cause of cancer-relevant deaths in the world. Carcinogenesis of HCC has several phases and complex processes. Several risk factors including: chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, cirrhosis, carcinogen exposure, and a group of single nucleotide polymorphisms, subscribe to hepatocarcinogenesis. Chronic HCV patients are sensitive to hepatic inflammation, liver fibrosis, cirrhosis, and HCC. But, it does not depend on the virus genotype or viral load (Samanoudy *et al.*, 2014). HBV is an important cause of chronic liver disease and the progression of liver fibrosis is still problematic like herpes virus infections among organ transplant patients in Iran (Yaghobi *et al.*, 2005; Mirzaee *et al.*, 2011). Histopathologically, liver fibrosis is an ongoing pathological complication in which several cellular and molecular events are involved. The formation of liver fibrosis is a dynamic progression, and is typically due to the degradation of cells synthesized extracellular matrix (ECM) proteins (Shin *et al.*, 2008). The main characteristic of fibrogenesis is exceeding accumulation of the ECM in liver tissue. Matrix metalloproteinases (MMPs) induce synthesis a matrix to control lower decomposition of connecting tissue proteins (Abdel-Latif, 2015).

MMPs are Zn^{2+} dependent extracellular endopeptidase enzymes play essential roles in the proteolysis of structural and signaling elements of the ECM and affect cell differentiation, proliferation, migration, and invasion, (Samanoudy *et al.*, 2014). MMPs are inflammatory indicators, which are critically important in the tissue destruction through physiologic and pathologic mechanisms. These mediators are produced by various cells from epithelial and mesenchymal sources (Akca *et al.*, 2013). Among them, MMP-2 has a role in remodeling and turnover of the basement membrane and tumor progression (Daniele *et al.*, 2014). MMP-2 gene (also known as gelatinase A) is placed on location 12.2 of chromosome 16 (Fanjul-Fernández *et al.*, 2010). Over expression of MMP-2 gene is seen in HCV related liver cirrhotic patients (Shackel *et al.*, 2002; Feng *et al.*, 2011). Increase in the levels of MMP-2 as hepatic gelatinases is associated with the fibrotic index in chronic HCV infected patients who have a history of development and recurrence of HCC (Abdel-Latif, 2015). Efforts now focus on optimization of the models to evaluate the role of MMPs in the progression of the liver cancers, develop and examine optimal intervention strategies, and affect the translation of therapies into the clinic. Therefore, in this study we were interested to find out whether there is any association between the expression level of MMP-2 and chronic HCV and HBV infections among liver transplanted patients.

MATERIALS AND METHODS

Patients

In this cross-sectional study 225 liver transplanted patients were recruited. These patients underwent surgery at Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran, between years 2012 and 2016. Formalin fixed paraffin embedded (FFPE) liver tissue sample was prepared. Blood samples were also collected in EDTA tubes. In this study samples from healthy individuals were also collected as normal controls. Ethical committee of Shiraz University of Medical Sciences approved the study design and protocols for sample preparation and analysis in compliance with the ethical guidelines of Helsinki Declaration.

As clinical procedure, those patients received the same routine regimens of immunosuppressive drugs including: cyclosporine or tacrolimus, with steroids and mycophenolate mofetile, were recruited in this study. The blood level of 200 mg/mL was considered as the therapeutic target for CsA (5mg/kg/d) or 10 mg/mL of tacrolimus (Afshari *et al.*, 2015; Karimi *et al.*, 2012).

HBV analysis

HBV immunologic Markers

HBV infection was confirmed by the presence of HBV immunologic markers including: HBsAg, HBeAg, HBeAb, and HBcAb in the plasma samples using 3rd generation ELISA kits (Diapro, Diagnostic-Italy) based on the instruction provided by the manufacturers.

HBV Molecular Assay

HBV genomic DNA was obtained from the patient en profile in the under tissue and plasma samples using DNA extraction Kit (CinnaGen, Tehran, Iran) according to the producer's instruction. The HBV-DNA was recognized in patient samples using particular primers to amplify a fragment of the surface gene using a qualitative HBV-PCR detection kit (CinnaGen, Tehran, Iran) according to the instructions of the producer (Zare *et al.*, 2016).

HCV Molecular Assay

The HCV-RNA genome was recognized in patients studied using nested RT-PCR protocol. First, using reverse transcriptase (derivative of Moloney murine leukemia virus) and random hexamer, cDNA was produced from 3µL of obtained HCV-RNA at 25°C for 60 minutes and 72°C for 10 minutes. The 20µL reverse transcription master mix included 0.2mmol of dNTPs, ribonuclease inhibitor (1 U/mL), random hexamer (0.01 mg/mL) and 4µL of the 5x reverse transcriptase buffer. Rounds of nested RT-PCR with specific primer pairs were used to amplify the 5-untranslated

regions HCV-RNA genome and the product size was 225 bp lengths (Mohammadi *et al.*, 2013). The HCV two step nested-RT-PCR program and primer sequences are presented in detail in Table 1 (Mirzaee *et al.*, 2012; Ebadi *et al.*, 2011).

MMP-2 Gene Expression

Total RNA was extracted using RNX plus solution (CinnaGen, Tehran, Iran). The quantity of RNA was measured using Nanodrop (OD:260/280). The extracted RNA quality was analyzed by running 3µL on 1% agarose gel. After achieving total RNA with good-quality, cDNA was produced using Prime Script RT reagent kit (Takara, Tokyo, Japan) based on the producer's guideline. By using SYBR Green Real-Time PCR method, the analysis of MMP-2 mRNA gene expression profile in the understudied patient groups and controls was performed. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control for minor fluctuations. The summary of PCR program and primer sequences are presented in Table 1. Melt curve was analyzed to confirm the specificity of reaction at the end of patient en profile in the under the program. To check the specificity of amplification reaction, melting-curve analysis was evaluated. The results for the target gene was measured as fluorescent signal intensity and compared with the internal standard gene GAPDH (Zare *et al.*, 2016).

Statistical analyses

The statistical discrepancy in the mRNA expression level of MMP-2 gene and the fold changes in viral infected patients and healthy controls were compared using the analysis of variance (ANOVA) and the Livak (ΔCT and $2^{-\Delta\Delta CT}$) methods. Statistical analyses were carried out using SPSS software (SPSS: An IBM Company, version 20). The $P \leq 0.05$ was considered significant.

RESULTS

The HBV group was consisted of 178 patients ranged from 4 to 67 years old (mean of 40.09 ± 14.4). The HCV group was included of 28 patients ranged from 21 to 62 years old (mean of 43.39 ± 11.28). The HBV and HCV co-infected group was also consisted of 19 patients ranged from 21 to 62 years old (mean of 44.05 ± 11.8). On the other hand, a healthy blood donor group consists of 20 persons ranged from 20 to 47 years old (mean of 33.6 ± 8.1) was added. The most frequent ABO blood group was O+ in all patient groups which are shown in Table 2. Patient demographics and laboratory liver tests for all studied patient groups are presented in Table 3.

Comparison of the mRNA expression level of MMP-2 gene in all patient groups versus controls

The mRNA expression level of MMP-2 gene showed a significant increase in all studied patient groups compared with controls using ΔCT analysis method (Figure 1). MMP-2 expression level was significantly increased in: HBV, HCV, and HBV with HCV co-infected patient groups compared with healthy controls (P=.000, 95%CI: 0.000-0.015; P=.000, 95%CI: 0.000-0.064; P=.000, 95%CI: 0.000-0.080), respectively.

Comparing the fold change of MMP-2 gene expression in all patient groups

The fold changes in MMP-2 gene mRNA expression levels were compared between all HBV, HCV, and HBV with HCV co-infected liver transplant patient groups using $2^{-\Delta\Delta CT}$ analysis method (Figure 2). The MMP-2 gene expression level was also increased more in HBV infected patients in comparing with HCV and HBV with HCV co-infected patients, but not significantly (Figure 2). MMP-2 gene expression level also shows 2.5 and 2.39 times increase in HBV infected patients compared with HCV and HBV with HCV co-infected ones, respectively. MMP-2 gene expression level was also increased 1.044 times more in HBV with HCV co-infected patients compared with HCV infected ones.

MMP-2 gene expression and risk factors

The results of the comparing between MMP-2 gene expression levels in all patient groups with different risk factors are presented in

Table 1. Polymerase Chain Reaction Condition of HCV, MMP-2 and GAPDH genes

Gene	Primer	Primer Sequence (5'-3')	PCR Product Length, base pair	Thermocycling Condition	PCR Mix
MMP-2	Forward Reverse	AAGGACAGCCCTGCAAGTTT GTCGTAGTCCTCAGTGGTGC	113	95°C/2min, 40 cycle at 95°C/15s, 58°C/20s, 72°C/30s, 95°C/15s, 58°C/60s, 95°C/15s	SYBR green Premix (5µl; 2× concentration), SYBR green dye (0.2µl; 50× concentration); forward Primer: 0.4µl and 5 pM; reverse primer 0.4µl and 5 pM
GAPDH	Forward Reverse	GGACTCATGACCACAGTCCA CCAGTAGAGGCAGGGATGAT	119		
HCV cDNA Synthesis				25°C/60min and 72°C/10min	5x RT buffer: 4µl, Random Hexamer: 1µl, 40u/µl RNase inhibitor: 0.5µl, 10mMdNTps: 2.5µl, 200u/µl M-Mulv: 0.75µl, D.W: 8.25µl, RNA: 3µl
HCV-PCR	Forward Reverse	CCCCTGTGAGGAACTACTGT CTGCACGGTCTACGAGACCTC		95°C/5min, 25 cycle at 94°C/50s, 55°C/40s, 72°C/50s, 72°C/3min	2µL cDNA, primers (0.1 pmol/L), deoxyribonucleoside triphosphate (0.2 mmol), Taq DNA polymerase (2.5U), 2.5µL of 10x PCR buffer, and magnesium chloride (1.5 mmol)
HCV-Nested PCR	Forward Reverse	CACGCAGAAAGCGTCTAGCCAT GTCGCAAGCACCCTATCAGGCAG	225	95°C/5min, 35 cycle at 94°C/40s, 64°C/35s, 72°C/40s, 72°C/3min	PCR buffer 10x: 2.5µl, Mgcl2 50Mm: 1.5µl, dNTps 10Mm: 0.5µl, PF 12.5 picomol/µl: 0.5µl, PR 12.5 picomol/µl: 0.5µl, Taq polymerase 5 unit/µl: 0.25µl, Water: 18.25µl, PCR-Product: 1µl

Abbreviations: GAPDH, glyceraldehydes-3-phosphate dehydrogenase; MMP-2, Matrix Metalloprotease-2.

Table 2. The Frequency of Blood Groups in HBV, HCV, and HBV with HCV co-infected liver trans-planted patient groups

Blood groups	HBV+ N (%)	HCV+ N (%)	HBV-HCV+ N (%)
A+	50(28.1%)	7(25%)	4(21.1%)
A-	1(0.6%)	–	–
B+	38(21.3%)	–	–
B-	2(1.1%)	–	–
AB+	22(12.4%)	4(14.3%)	3(15.8%)
AB-	2(1.1%)	–	–
O+	56(31.5%)	13(46.4%)	8(42.1%)
O-	7(3.9%)	4(14.3%)	4(21.1%)

Table 3. The underlying diseases in HBV, HCV, and HBV with HCV co-infected liver transplanted patient groups

Patients Underlying diseases	HBV+		HCV+		HBV-HCV+	
	Male N (%)	Female N (%)	Male N (%)	Female N (%)	Male N (%)	Female N (%)
Cryptogenic	55(41.99%)	25(53.19%)	12(60%)	5(62.5%)	–	2(40%)
HBV+	67(51.15%)	12(25.53%)	–	–	–	–
HCV+	–	–	5(25%)	2(25%)	–	–
HBV-HCV+	–	–	–	–	11(78.57%)	3(60%)
Autoimmiune Hepatitis	4(3.05%)	7(14.89%)	2(10%)	–	2(14.29%)	–
Wilson	3(2.29%)	–	–	–	–	–
Primary Sclerosing Cholangitis	2(1.53%)	2(4.26%)	1(5%)	–	1(7.14%)	–
Progressive Familial Intrahepatic Cholestasis	–	1(2.13%)	–	–	–	–
Buddchiari Syndrome	–	–	–	1(12.5%)	–	–
Total	131(73.6%)	47(26.4%)	20(71.43%)	8(28.57%)	14(73.68%)	5(26.32%)

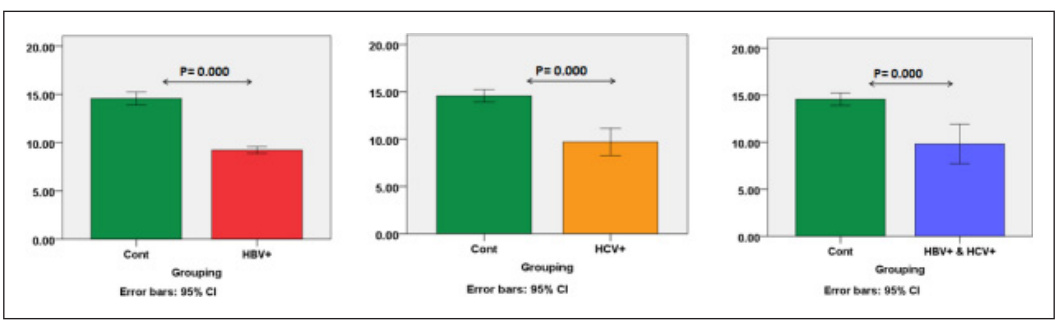

Figure 1. Comparing of the MMP-2 gene expression levels between HBV, HCV, and HBV with HCV co-infected liver transplant patient groups with controls. The expression level is measured based on ΔCt.

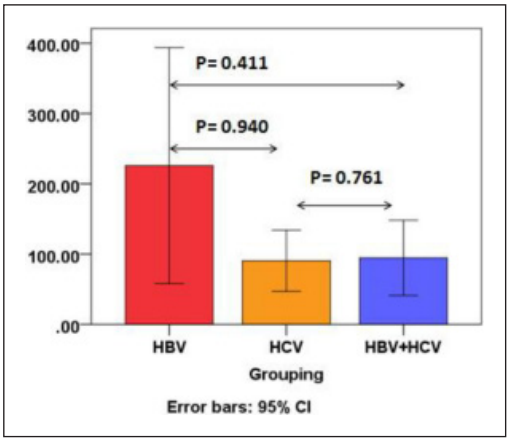

Figure 2. Comparing of the fold changes of the MMP-2 gene expression between HBV, HCV, and HBV with HCV co-infected liver transplant patient groups. The increasing fold of MMP-2 gene expression level is calculated using $2^{-\Delta\Delta Ct}$.

Table 4. No significant relationship was seen between the mRNA expression of MMP-2 gene and the studied risk factors.

DISCUSSION

HCC exhibits for 70-85% of the total liver cancer burden all around the world (Jemal *et al.*, 2011; Ferlay *et al.*, 2010). Each year more than 500,000 new patients are diagnosed with HCC worldwide (El-Serag 2011). Risk factors for HCC consist of cirrhosis with any etiology, alcohol intake, chronic HBV or HCV infections, aflatoxin exposure or metabolic disturbance. HBV is the major reason of acute hepatitis, cirrhosis, chronic hepatitis, and HCC, which affect more than 240 million people in the world. It is particularly prevalent in Africa, Asia, Latin America and Southern Europe (Liu *et al.*, 2014; Heim & Thimme, 2014). Approximately 30.0% of the world populations are conjectured to carry recognizable HBV antigens which results in more than half a million dead annually (Arzumanyan *et al.*, 2013; Westbrook and Dusheiko, 2014). Three percent of the people are also infected with HCV which is one of the most pressing emergencies worldwide (Webster *et al.*, 2015). It is estimated that 27.0% of the cases of cirrhosis and 25.0% of HCC cases worldwide can be attributed to HCV infection (Ott *et al.*, 2012).

One of the members of the gelatinase family is MMP-2 that recognized in the vascular areas of immature livers and has an essential role in the protection of liver vascular homeostasis, via its partnership in the TGF-β activation process (Ziaei *et al.*, 2014). In addition to the mediating TGF-β activation, MMP-2 is able to adjusting the activity of IL-1β, TNF-α, and MCP-3, via proteolytic cleavage (Duarte *et al.*, 2015).

The aim of this research was to determine the amount of MMP-2 gene expression level in viral hepatitis-infected liver transplanted patients and healthy controls. Results showed that MMP-2 expression level was meaningfully increased in all viral hepatitis

Table 4. Statistical relationships between expression of MMP-2 gene and risk factors

Indices		P value		
		HBV	HCV	HBV+HCV
1	Gender	0.495	0.488	0.494
2	Group	0.531	0.358	0.329
3	Pathology Underlying Disease	0.443	0.327	0.292
4	Clinical Underlying Disease	0.128	0.418	0.365
5	Recipient Age	0.114	0.306	0.331
6	Recipient Age Range	0.262	0.071	0.364
7	Recipient Blood Grouping	0.200	0.106	0.154
8	Donor Age	0.224	0.273	0.381
9	Donor Age Range	0.256	0.395	0.346
10	Donor Blood Grouping	0.925	0.670	0.559
11	Expired	0.421	0.386	0.364
12	Graft Rejection	0.318	0.386	0.364

infected liver transplant patients compared with the controls. MMP-2 gene expression level also showed increase in HBV infected patients compared with other viral hepatitis single and co-infected ones.

Studies showed the prognostic role of MMP-2 in HCC patients with the highest levels of MMP-2 at baseline before treatment (Daniele *et al.*, 2014). Giannelli *et al.* found a very significant correlation between higher serum levels of MMP-2 at baseline in the HBV and HCV infected patients (Giannelli *et al.*, 2002).

Wang and colleges also stated that MMP-2 is the most essential enzyme in the process of ECM remodeling which is involved in tumor invasion and metastasis, and could serve as an indicator for survival in patients with carcinoma in breast, head and neck, thyroid, superficial transitional cell and bladder (Wang & Wen 2012; Theret *et al.*, 2001). Another research claimed that MMP-2 and -9 are important proteases for migration and invasion incidents (Feng *et al.*, 2011). Also, their findings showed that the EMMPRIN-MMPs pathway was one of the mechanisms used by HCV core protein to elevate the migration of host cells (Fingleton, 2006). MMP promoters like MMP-2 and -9 harbor multiple cis-elements allowing for the regulation of MMP gene expression by a various set of trans-activators, containing Sp1 (Yan and Boyd, 2007). This process maybe the second mechanism used by HCV core protein to elevate the migration and invasion of host cells expect for more studies. Previous studies emphasized on the role of MMPs in the angiogenic switch which is one of the earliest stages of tumor growth and progression (Roy *et al.*, 2009). Other studies have documented that a meaningful positive relevance between serum MMP-2 and the stage of liver fibrosis exists in children with chronic HBV compared to the controls (Lebensztejn *et al.*, 2007). But, Reif *et al.* and Walsh *et al.* reported similar MMP-2 serum level in both patients and healthy controls. This finding is not in agreement with the data presented by Chen *et al.* (Reif *et al.*, 2005; Walsh *et al.*, 1999; Chen *et al.*, 2005) who displayed a higher level of MMP-2 among patients with hepatitis. These findings are also confirmed with others who also demonstrated a relevance of serum level of MMP-2 and -9 with fibrosis stage. Although, in contrast, Reif *et al.* realized that MMP-2 and -9 are indicators of inflammation but not the degree of fibrosis. Hepatic mRNA expression of MMP-1, -2, -3, -7, -9, -11, -13, and -14 were extremely variable in human liver with an increase of three logarithmic steps from lowest to the strongest expression of MMPs. Meaningful change in MMP-2 level during the course of chronic HCV were exhibited (Lichtinghagen *et al.*, 2003). Another study also reported that draining lymph nodes from tumor specimens exhibited meaningful increases in mRNA expression of MMP-2,-7, and -9 (Mckenna *et al.*, 2002). other study reported that MMP-2 is meaningfully motivated in cell cultures and also in the peripheral blood mononuclear cells of patients infected with HCV. These results are in accordance with those of previous researches exhibiting that MMP-2 is promoted in the serum of patients with chronic hepatitis, cirrhosis, and HCC (Ishii *et al.*, 2003; Li *et al.*, 2012).

Akca and associates determined that the maximum level of MMP-2 has been discovered in the HCV-LIA positive and HCV-RNA positive patients. This can be described by the patients who are previously diagnosed as HCV infected and are expected to be as HCV-RNA positive carriers, because they were under treatment for HCV infection (Akca *et al.*, 2013).

CONCLUSION

Finally, results of this study showed a significant increase in MMP-2 expression level in all viral hepatitis single and co-infected liver transplant patients in comparing with controls. More elevation of MMP-2 gene expression in HBV infected patients compared with other viral infected ones open a new window to deeply evaluate the role of MMP-2 in HBV pathogenesis in further completed studies.

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REFERENCES

Abdel-Latif, M.S. (2015). Plasma Levels of Matrix Metalloproteinase (MMP)-2, MMP-9 and Tumor Necrosis Factor-in Chronic Hepatitis C Virus Patients. *The Open Microbiology Journal* **9**: 136-140.

Akca, G., Tuncbilek, S. & Sepici-Dincel, A. (2013). Association between matrix metalloproteinase (MMP)-2, MMP-9 and total antioxidant status of patients with asymptomatic hepatitis C virus infection. *Letters in Applied Microbiology* **57**: 436-442.

Afshari, A., Yaghobi, R., Hossein Karimi, M., Darbouy, M., Azarpira, N., Geramizadeh, B., Malek-Hosseini, S.A. & Nikeghbalian, S. (2015). IL-17 mRNA Expression and Cytomegalovirus Infection in Liver Transplant Patients. *Experimental and Clinical Transplantation* **1**: 83-89.

Arzumanyan, A., Reis, H.M. & Feitelson, M.A. (2013). Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nature Reviews Cancer* **13**: 123-135.

Chen, T.Y., Hsieh, Y.S., Yang, C.C., Wang, C.P., Yang, S.F., Cheng, Y.W. & Chiou, H.L. (2005). Relationship between matrix metalloproteinase-2 activity and cystatin C levels in patients with hepatic disease. *Clinical Biochemistry* **38**: 632-638.

Daniele, A., Divella, R. & Mattioli, V. (2014) . Clinical and prognostic role of circulating MMP-2 and its inhibitor TIMP-2 in HCC patients prior to and after trans-hepatic arterial chemo-embolization. *Clinical Biochemistry* **47**: 184-190.

Duarte, S., Baber, J., Fujii, T. & Coito, A.J. (2015). Matrix metalloproteinases in liver injury, repair and fibrosis. *Matrix Biology* **44**: 147-156.

Ebadi, M., Yaghobi, R., Geramizadeh, B., Bahmani, M.K., Malek-Hosseini, S.A. & Nemayandeh, M. (2011). Prevalence of HCV and HGV Infections in Iranian Liver Transplant Recipients. *Transplantation Proceedings* **43**: 618-620.

El-Serag, H.B. (2011). Hepatocellular carcinoma. *The New England Journal of Medicine* **365**: 1118-1127.

Fanjul-Fernández, M., Folgueras, A.R., Cabrera, S. & López-Otín, C. (2010). Matrix metalloproteinases: Evolution, gene regulation and functional analysis in mouse models. *Biochimica et Biophysica Acta* **1803**: 3-19.

Feng, X., Xiu, B., Xu, L., Yang, X., He, J., Leong, D., He, F. & Zhang, H. (2011). Hepatitis C virus core protein promotes the migration and invasion of hepatocyte via activating transcription of extracellular matrix metalloproteinase inducer. *Virus Research* **158**: 146-153.

Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C. & Parkin, D.M. (2010). Estimates of worldwide burden of cancer in 2008:GLOBOCAN 2008. *International Journal of Cancer* **127**: 2893-917.

Fingleton, B. (2006). Matrixmetalloproteinases: roles in cancer and metastasis. *Frontiers in Bioscience* **11**: 479-491.

Giannelli, G., Bergamini, C., Marinosci, F., Fransvea, E., Quaranta, M., Lupo, L., Schilardi, O. & Antonaci, S. (2002). Clinical role of MMP-2/TIMP-2 imbalance in hepatocellular carcinoma. *International Journal of Cancer* **97**: 425-31.

Heim, M.H. & Thimme, R. (2014). Innate and adaptive immune responses in HCV infections. *Journal of Hepatology* **61**: 14-25.

Ishii, Y., Nakasato, Y. & Kobayashi, S. (2003). A study on angiogenesis-related matrix metalloproteinase networks in primary hepatocellular carcinoma. *Journal of Experimental & Clinical Cancer Research* **22**: 461-470.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. & Forman, D. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians* **61**: 69-90.

Karimi, M.H., Maryam Motazedian, M., Abedi, F., Yaghobi, R., Geramizadeh, B. & Nikeghbalian, S. (2012). Association of genetic variation in co-stimulatory molecule genes with outcome of liver transplant in Iranian patients. *Gene* **504**: 127-132.

Lebensztejn, D.M., Skiba, E., Sobaniec-Łotowska, M.E. & Kaczmarski, M. (2007). Matrix metalloproteinases and their tissue inhibitors in children with chronic hepatitis B treated with lamivudine. *Advances in Medical Sciences* **52**: 114-119.

Leroy, V., Monier, F., Bottari, S., Trocme, C., Sturm, N., Hilleret, M.N., Morel, F. & Zarski, J.P. (2004). Circulating matrix metalloproteinases 1,2,9 and their inhibitors TIMP-1 and TIMP-2 as serum markers of liver fibrosis in patients with chronic hepatitis C: Comparison with PIIINP and hyaluronic acid. *The American Journal of Gastroenterology* **99**: 271-9.

Li, Y., Zhang, Q., Liu, Y., Luo, Z., Kang, L., Qu, J., Liu, W., Xia, X., Liu, Y., Wu, K. & Wu, J. (2012). Hepatitis C Virus Activates Bcl-2 and MMP-2 Expression through Multiple Cellular Signaling Pathways. *Journal of Virology* **86**: 12531-12543.

Lichtinghagen, R., Bahr, M.J., Wehmeier, M., Michels, D., Haberkorn, C.I., Arndt, B., Flemming, P., Manns, M.P. & Boeker, K.H.W. (2003). Expression and co-ordinated regulation of matrix metalloproteinases in chronic hepatitis C and hepatitis C virus-induced liver cirrhosis. *Clinical Science* **105**: 373-382.

Liu, Y., Wang, X., Li, S., Hu, H., Zhang, D., Hu, Peng. & Ren, H. (2014). The role of von Willebrand factor as a biomarker of tumor development in hepatitis B virus-associated human hepatocellular carcinoma: A quantitative proteomic based study. *Journal of Proteomics* **106**: 99-112.

McKenna, G.J., Chen, Y., Smith, R.M., Meneghetti, A., Ong, C., McMaster, R., Scudamore, C.H. & Chung, S.W. (2002). A role for matrix metalloproteinases and tumor host interaction in hepatocellular carcinomas. *The American Journal of Surgery* **183**: 588-594.

Mirzaee, M., Yaghobi, R., Ramzi, M. & Roshan Nia, M. (2012). The prevalence of molecular and immunologic infective markers of hepatitis viruses in patients with hematological malignancies. *Molecular Biology Reports* **39**: 1217-1223.

Mohammadi, B., Yaghobi, R., Dehghani, M. & Behzad Behbahani, A. (2013). The Molecular Prevalence of Viral Infections in Transplant Candidates with Bone Marrow Suppression, Shiraz, Southern Iran, 2010. *International Journal of Organ Transplantation Medicine* **4**(2): 87-94.

Ott, J.J., Stevens, G.A., Groeger, J. & Wiersma, S.T. (2012). Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* **30**: 2212-2219.

Reif, S., Somech, R., Brazovski, E., Reich, R., Belson, A., Konikoff, F.M. & Kessler, A. (2005). Matrix metalloproteinase 2 and 9 are markers of inflammation but not of the degree of fibrosis in chronic hepatitis C. *Digestion* **71**: 124-30.

Roy, R., Yang, J. & Moses, M.A. (2009). Matrix Metalloproteinases As Novel Biomarkers and Potential Therapeutic Targets in Human Cancer. *Journal of Clinical Oncology* **27**: 5287-5297.

Samanoudy, A.El., Monir, R., Badawy, A., Ibrahim, L., Farag, K., Baz, S.El., Alenizi, D. & Alenezy, A. (2014). Matrix metalloproteinase-9 gene polymorphism in hepatocellular carcinoma patients with hepatitis B and C viruses. *Genetics and Molecular Research* **13**: 8025-8034.

Shackel, N.A., McGuinness, P.H., Abbott, C.A., Gorrell, M.D. & McCaughan, G.W. (2002). Insights into the pathobiology of hepatitis C virus-associated cirrhosis: analysis of intrahepatic differential gene expression. *American Journal of Pathology* **160**: 641-654.

Shin, H.P., Lee, J.I., Jung, J.O., Yim, S.V., Kim, H.J., Cha, J.M., Park, J.B., Joo, K.R., Hwang, J.S. & Jang, B.K. (2008). Matrix Metalloproteinase (MMP)-3 Polymorphism in Patients with HBV Related Chronic Liver Disease. *Springer* **53**: 823-829.

Theret, N., Musso, O. & Turlin, B. (2001). Increased extracellular matrix remodeling is associated with tumor progression in human hepatocellular carcinomas. *Hepatology* **34**: 82-88.

Walsh, K.M., Timms, P., Campbell, S., MacIver, R.N. & Morris, A.J. (1999). Plasma level of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinase-1 and -2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C. Comparison using ROC analysis, *Digestive Diseases and Sciences* **44**: 624-30.

Wang, H. & Wen, W. (2012). Biomarkers of Hepatocellular Carcinoma. *Hangzhou and Springer* 79-154.

Webster, D.P., Klenerman, P. & Dusheiko, G.M. (2015). Hepatitis C. *Lancet* **385**: 1124-1135.

Westbrook, R.H. & Dusheiko, G. (2014). Natural history of hepatitis C. *Journal of Hepatology* **61**: S58-68.

Yaghobi, R., Behzad-Behbahani, A., Sabahi, F., Roustaee, M.H., Alborzi, A., Ramzi, M. & Nourani, H. (2005). Comparative analysis of a double primer PCR assay with plasma, leukocytes and antigenemia for diagnosis of active human cytomegalovirus infection in bone marrow transplant patients. *Bone Marrow Transplantation* **35**: 595-599.

Yan, C. & Boyd, D.D. (2007). Regulation of matrix metalloproteinase gene expression. *Journal of Cellular Physiology* **211**: 19-26.

Zare, A.H., Karimi, M.H., Rashki, A., Geramizadeh, B., Afshari, A., Miri, H.R. & Yaghobi, R. (2017). Association of the Interleukin-27 Gene Expression and Hepatitis B Virus Infection in Liver Transplanted Patients. *Experimental and Clinical Transplantation* **5**: 554-560.

Ziaei, R., Ayatollahi, M., Yaghobi, R., Sahraeian, Z. & Zarghami, N. (2014). Involvement of TNF-α in differential gene expression pattern of CXCR4 on human marrow-derived mesenchymal stem cells. *Molecular Biology Report* **41**: 1059-1066.