Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Association between matrix metalloproteinase (MMP)-2, MMP-9 and total antioxidant status of patients with asymptomatic hepatitis C virus infection

G. Akca[1], S. Tunçbilek[2] and A. Sepici-Dinçel[3]

1 Department of Microbiology, Faculty of Dentistry, Gazi University, Ankara, Turkey
2 Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, Ufuk University, Ankara, Turkey
3 Faculty of Medicine, Gazi University, Ankara, Turkey

Significance and Impact of Study: Hepatitis C virus (HCV) is the most studied viral agent and the common cause of the chronic liver diseases. It is generally asymptomatic and causes hepatic inflammation and tissue damage. Here, in this study, the potential hepatocellular damage due to the HCV was predicted by different metabolic pathways' biochemical markers such as total antioxidant status, matrix metalloproteinase (MMP)-2 and MMP-9. This manuscript may not only raise awareness in dental patients, who are asymptomatic for HCV infection but also help predict any potential damage in liver tissue without using an invasive diagnostic method even if the patients have normal alanine aminotransferase and aspartate aminotransferase records.

Keywords
alanine aminotransferase, aspartate aminotransferase, asymptomatic infection, hepatitis C virus, matrix metalloproteinase-2, matrix metalloproteinase-9, quantitative PCR, total antioxidant status.

Correspondence
Gülçin Akca, Department of Microbiology, Faculty of Dentistry, Gazi University, 82. sk no:406510, Ankara, Turkey
E-mail: gulcin68@yahoo.com

2013/1052: received 29 May 2013, revised 24 June 2013 and accepted 3 July 2013

doi:10.1111/lam.12131

Abstract

The aim of this study is to predict potential hepatocellular damage by determining total antioxidant status (TAS) and matrix metalloproteinases (MMPs) 2 and 9 levels of different groups of dental surgery patients who are asymptomatic (normal alanine aminotransferase, aspartate aminotransferase records). Patients were divided into five groups according to the anamnesis [to be diagnosed formerly as hepatitis C virus (HCV) infection or not], microbiological (positive-anti-HCV antibodies and HCV RNA-positive or negative) and biochemical test results. Except for the control group, serum anti-HCV antibody levels and line immunoassay tests were found positive in all groups. HCV RNAs were found positive only in group 3 whom were formerly diagnosed with HCV infection, not under medical treatment and in group 5 under medical treatment ($<2 \times 10^5$ IU ml^{-1}). Statistical analyses were performed using one-way multifactorial ANOVA (MANOVA) at the statistical significance level of 5% and were confirmed that the changes in biochemical markers had significant effects on subjects who had been in different groups. Following multiple comparisons, significant groups' differences were obtained in all biochemical markers. In conclusion, to determine not only TAS levels but also the MMPs and evaluate those together may be noninvasive biomarkers for predicting the inflammation in liver and approaching the prognosis of HCV infection.

Introduction

Due to the gradual increase in the role played in chronic hepatitis cases, cirrhoses and hepatocellular carcinoma (Osella *et al.* 2001), hepatitis C virus (HCV) is still one of the most studied viral agents. HCV infection results in liver injury and long-term complications; yet, they are generally asymptomatic and cannot be diagnosed with routine biochemical tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT)]. Eighty-five per cent of the infected individuals develop persistent chronic infection and chronic hepatitis (Lauer and Walker 2001).

Liver biopsy is still considered as the golden standard. However, this procedure cannot be applied to everybody since it is invasive and causes bleeding. Usually, it is preferred to use noninvasive, easier and cheaper biomarkers to follow the prognosis of the asymptomatic patients.

Determination of anti-HCV antibodies by ELISA method is the most commonly used diagnostic test method. However, under some circumstances, it is possible to see false seropositive records. Besides, due to the late formation of anti-HCV antibodies, especially during the acute phase, this test is not always considered satisfactory and reliable alone; and therefore, supplementary diagnostic reconfirmation tests [line immunoassay and quantitative PCR (qPCR)] are required to validate viremia and prognosis of infection.

Fibrosis and cirrhosis develop during the course of chronic liver disease. Several biochemical indicators have been reported as potential noninvasive serum/plasma markers of fibroproliferation. Among them, the matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been shown by several groups to correlate in some way with the development of cirrhosis, the extent of toxic damage to the liver in alcoholic liver disease and the inflammatory activity in patients with chronic viral hepatitis (Kasahara *et al.* 1997; Walsh *et al.* 1999; Böker *et al.* 2000). Until present, 28 extracellular MMPs classes have been presented as inflammatory markers, which play a role in tissue degradation by physiological and pathological mechanisms. They are synthesized from various cells of epithelial and mesenchymal origin, including leucocytes, keratinocytes, fibroblasts, macrophages, chondrocytes and smooth muscle cells. MMPs, through their proteolytic activity, play crucial roles in invasion and metastasis; interfere with signalling pathways controlling cell growth, survival, invasion, inflammation and angiogenesis (Laack *et al.* 2002; Yang *et al.* 2005).

In addition, HCV infections are also thought of causing enhanced oxidative stress associated with hepatic free radical formation (Ferre *et al.* 2005). The increased production of reactive oxygen species (ROS) depends on an imbalance of oxidant/antioxidant systems and the destructive chain reaction initiated by ROS can be terminated by antioxidants. (Sies 1997; Kohen and Nyska 2002).

The aim of this study is to predict potential hepatocellular damage due to the HCV by determining total antioxidant status (TAS), MMP-2 and MMP-9 levels of the patients who are asymptomatic for this infection and can be transmitted by HCV via any possible inappropriate way of transmission. Thereby, it is thought that this report may not only raise awareness in dental patients, who are asymptomatic for HCV infection but also help predict any potential damage in liver tissue without using an invasive diagnostic method even if the patients have normal ALT and AST records.

Results and discussion

In this study, our main objective was to suggest noninvasive predictors as indicative of liver damage in HCV-infected patients or patients susceptible of being infected by HCV. Together with conventional biochemical markers, such as ALT and AST levels, present work focuses in two putative predictors, the levels of TAS, MMPs 2 and 9 analysed in the serum of asymptomatic patients who were planned to undergo dental surgery.

Currently, it was reported that three to four million people have been newly infected by HCV each year (Bellentani *et al.* 2000; Shepard *et al.* 2005). In Turkey, although HCV seroprevalence has been considered to be low (%0·3–1·8), it was estimated that approximately 700 000 people had been affected (Karaca *et al.* 2006). Unfortunately, because most of them are asymptomatic, hepatitis C is rarely identified or reported. On the other hand, although the transmission of HCV due to dental needle injury is reported to be as low as 3% (Scully and Greenspan 2006; Tarantola *et al.* 2006), dental practitioners and their patients are highly exposed to HCV since HCV transmission may occur via directly saliva or contaminated dental instruments that comprises micro/macro amount of blood. In addition, respiratory and permucosal transmission is accounted for the other ways of contamination. Thus, determining the presence of viral agents with the easy and cost-effective methods in every hospital's microbiology and biochemistry laboratories would be an initial step for further protective measures. Determination and confirmation of anti-HCV antibody presence using molecular techniques such as qPCR validate the diagnosis of the infected individual, who poses the risk and capacity of transmission. But under the circumstances that HCV RNA was found negative, the prognosis of infection and the status of tissue damage has been thought to be unclear. In our study, except for the control group, serum anti-HCV antibody levels and line immunoassay tests were found positive in all groups. But HCV RNAs were found positive only in patients of groups 3 and 5 ($<2 \times 10^5$ IU ml^{-1}). In acute HCV infection, significantly higher ALT levels have been found in HCV-infected patients but in chronic state, they were mostly within the normal scores. The descriptive statistics of biochemical parameters of our study; AST (U l^{-1}), ALT (U l^{-1}), TAS (mmol l^{-1}), MMP-2 (ng ml^{-1}), MMP-9 (ng ml^{-1}) among groups were presented in Table 1. A one-way MANOVA revealed a significant multivariate main effect for groups, Wilks' $\lambda = 0.019$, $F(20, 173{\cdot}4) = 19{\cdot}965$, $P = 0.0001$, partial eta squared = 0.629.

Table 1 Serum AST (U l^{-1}), ALT (U l^{-1}), TAS (mmol l^{-1}), MMP-2 (ng ml^{-1}) and MMP-9 (ng ml^{-1}) levels of all groups

Groups	Serology	Molecular tests		Biochemical parameters (mean ± SD)				
	Anti-HCV Ab	HCV LIA	HCV RNA	ALT (U l^{-1})	AST (U l^{-1})	TAS (mmol l^{-1})	MMP-2 (ng ml^{-1})	MMP-9 (ng ml^{-1})
Group 1 Control (n = 10)	Negative	Negative	Negative	12·30 ± 3·43	12·20 ± 1·81	1·94 ± 0·03	457·41 ± 116·72	Not detectable
Group 2 (n = 13)	Positive	Positive	Negative	11·92 ± 1·18	15·38 ± 6·72	1·08 ± 0·58	386·10 ± 56·63	276·85 ± 59·10
Group 3 (n = 15)	Positive	Positive	Positive	13·66 ± 3·82	15·80 ± 7·36	1·06 ± 0·48	990·11 ± 45·71	64·51 ± 6·21
Group 4 (n = 13)	Positive	Positive	Negative	11·75 ± 1·60	14·16 ± 4·62	1·18 ± 0·49	495·08 ± 118·03	159·90 ± 18·05
Group 5 (n = 10)	Positive	Positive	Positive	35·70 ± 29·70	40·00 ± 18·93	0·91 ± 0·59	684·59 ± 59·33	96·64 ± 5·46

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; MMP, matrix metalloproteinase; TAS, total antioxidant status.

Power to detect the effect was 1. Thus, it was confirmed that the changes in biochemical markers had significant effects on subjects who had been in different groups, which were enrolled into the study. Given the significance of the overall test, the univariate main effects were examined. Significant univariate main effects for groups were obtained for ALT, $F(4, 56) = 7{\cdot}57$, $P = 0.0001$, partial eta square = 0.351, power = 0.995; for AST, $F(4, 56) = 12{\cdot}89$, $P = 0.0001$, partial eta square = 0.479, power = 1; for TAS, $F(4, 56) = 7{\cdot}94$, $P = 0.0001$, partial eta square = 0.362, power = 0.996; for MMP-2, $F(4, 56) = 8{\cdot}37$, $P = 0.0001$, partial eta square = 0.374, power = 0.998; for MMP-9, $F(4, 56) = 161{\cdot}5$, $P = 0.0001$, partial eta square = 0.92, power = 1. Following multiple comparisons, significant groups' differences were obtained in ALT, AST, TAS, MMP-2 and MMP-9 (Table 2). According to the statistical analyses, in comparison with groups 1, 2, 3, 4 with group 5, ALT ($P < 0{\cdot}001$) and AST ($P < 0{\cdot}05$) levels were found statistical significant even if they were within the test intervals. On the other hand, the highest ALT and AST levels were found in chronic asymptomatic HCV patients (group 5), and this group was found to be statistically significantly different from all of the groups in the study including the control group.

In accord with our findings, it was also reported that ALT flare-ups of the patients were associated with increased levels of oxidative stress markers. Besides, it was suggested that increased oxidative stress in liver tissue during disease activation might reflect a tendency of decreased plasma TAS (Vendemiale *et al.* 2001). Our results indicated statistically significant decreased TAS levels ($P \leq 0{\cdot}001$) in all of the groups, in comparison with the control group, which was found to be in line with the study of Vendemiale *et al.* 2001;. The minimum TAS levels were found in chronic asymptomatic HCV patients who might have still been under surveillance and taking some medications. None of our patients in the control

Table 2 Tukey significant multiple comparison test results for the differences of biochemical markers among groups

Variables	Groups		Significance	Confidence interval	
				Lower	Upper
ALT	2	5	0·0001	−38·2	−9·3
	3	5	0·0001	−36·01	−8
	4	5	0·0001	−37·9	−9·1
	1	5	0·001	−38·76	−8·03
AST	2	5	0·0001	−36·61	−12·62
	3	5	0·0001	−35·84	−12·55
	4	1	0·047	−24·08	−0·08
	4	5	0·0001	−37·38	−13·38
	1	5	0·037	−26·5	−0·5
TAS	2	1	0·001	−1·4	−0·3
	3	1	0·0001	−1·42	−0·33
	4	1	0·0001	−1·45	−0·32
	1	5	0·0001	0·44	1·63
MMP-2	2	3	0·0001	−583·18	−171·33
	2	5	0·0001	−495·8	−38·64
	3	4	0·006	55·7	467·55
	3	1	0·004	81·4	525·11
MMP-9	2	3	0·0001	181·51	242·9
	2	4	0·0001	84·46	148·1
	2	1	0·0001	242·67	310·91
	2	5	0·0001	146·03	214·27
	3	4	0·0001	−126·73	−65·23
	3	1	0·0001	31·41	97·64
	4	1	0·0001	126·38	194·62
	4	5	0·0001	29·74	97·98
	1	5	0·0001	−132·92	−60·36

ALT, alanine aminotransferase; AST, aspartate aminotransferase; MMP, matrix metalloproteinase; TAS, total antioxidant status.

group had positive results from the HCV RNA assays, which was accepted as a finding also supporting the hypothesis that a relation between oxidative stress and HCV infection may not become clinically significant in patients with low disease activity and normal ALT levels. In another study, it was reported that levels of antioxidant agents increased in the serum of HBV- or HCV-infected uraemic patients, and they concluded that increased plasma antioxidant capacity might reflect oxidative injury in liver tissue (Pawlak *et al.* 2004). On the other hand, clinically inactive HCV infection was reported to be associated with reduced oxidative stress, compared with active HCV infection or HCV-negative patients undergoing haemodialysis (Sezer *et al.* 2006; Tutal *et al.* 2010). These results were not completely in agreement with ours. The most possible cause of this paradox could be the differences in patient groups of the two studies since their patients were uraemic or receiving haemodialysis. The authors suggested that this may be a reactionary decrease to increased oxidative load at the tissue level; or secondary to decreased liver biosynthetic activity. Plasma or serum TAS has been shown to be directly related to complications. The altered concentrations of antioxidants suggest that the defence system is active and effective, with lower levels showing depletion due to utilization. TAS levels of body fluids are directly related to antioxidant levels and free radical production. In our study, we showed that the presence of chronic HCV infection was associated with decreased plasma antioxidant status. However, higher antioxidant status was observed in HCV RNA-negative patients, in comparison with HCV RNA-positive subjects. TAS contends the cumulative action of all the antioxidants present in plasma and body fluids, thus providing an integrated parameter in a short period of time with less cost than the sum of all measurable antioxidants. It is presently the preferred and widely used method for the assessment of oxygen radical absorbance status capable of protecting the cells via inhibition of oxidant reactions (Wang *et al.* 2001). Therefore, TAS may act as a protective antioxidant to combat the metabolic oxidative stress-induced.

It is known that core protein of HCV can also trigger the inflammatory cells (Dolganiuc *et al.* 2003; Nattermann *et al.* 2005) Inflammatory processes are also known to promote oxidative stress and increase ROS production in the organism and are therefore regarded as one of the major causes of inflammation. The micro-environmental tissue damage has been linked to the release of ROS, and reactive species, which are released by activated neutrophils can easily attack almost every cell component. Moreover, environment, sex, age, hormones, lifestyle and dietary factors are all potential factors involved in the redox modulation of humans (Halliwell and Whiteman 2004). In our study, we used MMP-2 and MMP-9 markers to discuss the inflammatory status of patients. MMP-2 (gelatinase A, collagenase IV) and MMP-9 (gelatinase B, collagenase 3) are proteolytic enzymes, which are responsible for the degradation of Type IV collagen and change intact status of liver tissue eventually leading to liver damage. Besides, they were linked to causation of invasion into tissues and correlated with tumour aggressiveness and metastatic potential. (Miller *et al.* 1993; Wang *et al.* 2001; Laack *et al.* 2002; Yang *et al.* 2005). They have been activated during hepatic fibrogenesis and contributed to liver damage. On the other hand, MMPs play an important role in orchestrating leucocyte extravasation into the inflammatory focus (Shukla *et al.* 2012). So, they facilitate penetration of inflammatory cells into the liver and enhance local tissue damage.

The highest levels of MMP-2 have been found in the study group 3. In comparison with group 3 with other groups (1, 2 and 4), statistical significant decrease was found between the MMP-2 levels of other groups. This can be explained by these patients who are formerly diagnosed as HCV infection and supposed to be as carriers (positive HCV RNA), since they have been kept under medical surveillance for HCV infection. MMP-2 levels of group 2 was found statistically significant in comparison with the levels of chronic asymptomatic HCV patients who have the highest ALT and AST levels among the patients in other groups. This can be explained by invasion of this agent into the liver tissue and the status of the inflammation. Due to several cofactors (age, diet, obesity, lifestyle, alcohol use, lack of medical controls etc.), MMP-2 levels of these patients might be affected. Therefore, these patients are not needed to be followed or to take any medication.

On the contrary, the highest levels of MMP-9 have been found in patients of group 2 as they were healthy and negative for HCV RNA and even if they were not as much as MMP-2 levels. For MMP-9 biomarker, only in HCV-free dental patients, MMP-9 levels were found undetectable as expected in healthy people. According to this biomarker, in comparison all of the groups with each other, statistically significant difference was found in each of the groups ($P = 0{\cdot}0001$) except between the groups 3 and 5.

Especially, in comparison with the chronic asymptomatic patients with other groups', it was found that MMP-9 might play an important role for predicting the status of liver damage and inflammation. As the scores were found statistically significant even if the ALT and AST levels were within normal intervals, and the patients were clinically asymptomatic for HCV. This leads us to conclude that MMP-9 may be the major and/or key

modulator of signalling pathway regulation, which is responsible for invasion of cells.

In conclusion, the mechanisms by which HCV causes cell damage still need to be elucidated and clearly defined. Based on the findings of this study, we may suggest the use of the selected biomarkers in monitoring the status of patients, in the determination of prognosis and in the management of HCV infection. Furthermore, even if the patients are asymptomatic, these biomarkers would provide necessary data for predicting the level of the damage in liver tissue. Hereby, to determine not only TAS levels but also the MMPs and evaluate these records together may be a noninvasive approach for predicting the inflammation in liver, and the role of HCV in pathophysiology of infection and its comorbidity. On the other hand, there might be several cofactors (age, diet, obesity, lifestyle, overuse of alcohol etc.) that may influence the results of the study. Therefore, further studies with larger sample size are imperative to define the role of MMPs and TAS biomarkers for evaluating liver damage.

Material and methods

The patients who are planned to undergo dental surgery in Gazi University Faculty of Dentistry were enrolled in this study. An informed consent was obtained from all the subjects, and the research was made according to Helsinki Declaration. Before surgery, for standard preoperative procedures, serum of the patients was taken for routine haematologic, biochemical and microbiological tests to apply premedication. Tests in this study were also run routinely on these patients' serum. Patients ($n = 61$) were divided into five groups: The first group (Group 1, $n = 10$) includes individuals who will undergo various dental procedures with the test results as anti-HCV antibodies are negative and studied as the control group. Their results were also assessed by line immunoassay and qPCR and found to be negative. Second group (Group 2, $n = 13$) includes asymptomatic patients who are formerly diagnosed with HCV infection but not under medical treatment until present, with the test results as anti-HCV antibodies and HCV line immunoassay tests are positive, but HCV RNAs are negative. The third group (Group 3, $n = 15$) includes asymptomatic patients who are formerly diagnosed with HCV infection but not under medical treatment with the test results as anti-HCV antibodies, HCV line immunoassay tests and HCV RNAs are positive. The forth group (Group 4, $n = 13$) includes asymptomatic patients who are never diagnosed with HCV infection before with the test results as anti-HCV antibodies and line immunoassay tests are positive, but HCV RNAs are negative. The fifth group (Group 5, $n = 10$) includes asymptomatic chronic HCV patients, who are formerly diagnosed with HCV infection and medicated, with the test results as anti-HCV antibodies, line immunoassay tests and HCV RNAs are positive.

Microbiological, serological and molecular methods

Anti-HCV antibodies were detected by the ELISA tests carried out using the commercial 4th generation anti-HCV enzyme immunoassay kit (cat: no 4NAE3; General Biological Co, kaohsiung, Taiwan), and the results were calculated spectrophotometrically from optical densities (ELx800; BioTek, Winooski, VT, USA). For the correction of false seropositiveness of anti-HCV positive results, line immunoassay was performed using the 'strip design INNO-LIA™ HCV Score' (Innogenetics NV, Headquarters, Gent, Belgium) kit. This kit was used for utilizing well-defined antigens derived from HCV immunodominant proteins from the core region, the E2 hypervariable region (HVR), the NS3 helicase region and the NS4A, NS4B and NS5A regions. The antigens used are either recombinant proteins or synthetic peptides, highly purified and fixed on a nylon membrane. For discriminating acute and occult HCV infection, qPCR was carried out using Artus HCV RG-PCR kit (Qiagen, Venlo, the Netherlands). HCV RNA was isolated using the QIAamp DSP Virus Kit (Qiagen, Venlo, the Netherlands), and analysis was carried out on the Rotor-Gene 6000 Instrument (Qiagen, Hilden, Germany). The numbers of HCV RNA were defined as copy ml^{-1}.

Biochemical procedures

AST (U l^{-1}) and ALT (U l^{-1}) levels were determined with a clinical chemistry analyser (Roche/Hitachi Modular Analytics System, Modular P800 Module; Roche Diagnostics, Basel, Switzerland) and commercial kits. The matrix metalloproteinase-2 (MMP-2; ng ml^{-1}) and matrix metalloproteinase-9 (MMP-9; ng ml^{-1}) (Human, Biotrak™ ELISA System from GE Healthcare, Uppsala, Sweden) assays were based on a two site ELISA 'sandwich' format.

Total antioxidant status determination (TAS assay)

Plasma TAS (mmol l^{-1}) was measured with Randox commercial kit (Randox Laboratories Ltd., Crumlin, UK). The principle of the assay is incubating 2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) with ferryl-myoglobin radical, formed by the activation of a peroxidase (metmyoglobin) with hydrogen peroxide (H_2O_2), to produce the radical cation $ABTS^+$. This has a relatively stable blue–green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree proportional to their concentrations (Miller *et al.* 1993).

Statistical analysis

A one-way multivariate analysis of variance (MANOVA) was used to determine whether the biochemical markers influenced the groups of patients in the study. When MANOVA indicated this influence, a one-way analysis of variance was carried out to verify, which groups were influenced by biochemical markers. For this purpose, the Tukey multiple comparison test was used to identify the differences among groups. For the tests performed, the significance level was set at (α) 0·05. All the tests were performed with a statistical software package (SPSS V21 for Macintosh; IBM, Chicago, IL, USA).

Conflicts of interest

All authors disclose that they have no financial or personal relationships with other people or organizations. There is no direct financial interest in the subject matter or materials discussed in the manuscript that could inappropriately influence the work submitted. Investigators also disclose no potential conflicts to participants in clinical trials and other studies and state in the manuscript whether they have done so.

References

Bellentani, S., Miglioli, L., Masutti, F., Saccoccio, G. and Tiribelli, C. (2000) Epidemiology of hepatitis C virus infection in Italy: the slowly unraveling mystery. *Microbes Infect* **2**, 1757–1763.

Böker, K.H., Pehle, B., Steinmetz, C., Breitenstein, K., Bahr, M. and Lichtinghagen, R. (2000) Tissue inhibitors of metalloproteinases in liver and serum/plasma in chronic active hepatitis C and HCV-induced cirrhosis. *Hepatogastroenterology* **47**, 812–819.

Dolganiuc, A., Kodys, K., Kopasz, A., Marshall, C., Do, T., Romics, L., Mandrekar, P., Zapp, M. *et al.* (2003) Hepatitis C virus core and nonstructural protein 3 proteins induce pro- and anti-inflammatory cytokines and inhibit dendritic cell differentiation. *J Immunol* **170**, 5615–5624.

Ferre, N., Marsillach, J., Camps, J., Rull, A., Coll, B., Tous, M. and Joven, J. (2005) Genetic association of paraoxonase-1 polymorphisms and chronic hepatitis C virus infection. *Clin Chim Acta* **361**, 206–210.

Halliwell, B. and Whiteman, M. (2004) Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol* **142**, 231–255.

Karaca, C., Cakaloglu, Y., Demir, K., Ozdil, S., Kaymakoglu, S., Badur, S. and Okten, A. (2006) Risk factors for the transmission of hepatitis C virus infection in the Turkish population. *Dig Dis Sci* **51**, 365–369.

Kasahara, A., Hayashi, N., Mochizuki, K., Oshita, M., Katayama, K., Kato, M., Masuzawa, M., Yoshihara, H. *et al.* (1997) Circulating matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 as serum markers of fibrosis in patients with chronic hepatitis. *J Hepatol* **26**, 574–583.

Kohen, R. and Nyska, A. (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* **30**, 620–650.

Laack, E., Köhler, A., Kugler, C., Dierlamm, T., Knuffmann, C., Vohwinkel, G., Niestroy, A., Dahlmann, N. *et al.* (2002) Pretreatment serum levels of matrix metalloproteinase-9 and vascular endothelial growth factor in non-small-cell lung cancer. *Ann Oncol* **13**, 1550–1557.

Lauer, G.M. and Walker, B.D. (2001) Hepatitis C virus infection. *N Engl J Med* **345**, 41–52.

Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. and Milner, A. (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci (Lond)* **84**, 407–412.

Nattermann, J., Nischalke, H.D., Hofmeister, V., Ahlenstiel, G., Zimmermann, H., Leifeld, L., Weiss, E.H., Sauerbruch, T. *et al.* (2005) The HLA-A2 restricted T cell epitope HCV core 35-44 stabilizes HLA-E expression and inhibits cytolysis mediated by natural killer cells. *Am J Pathol* **166**, 443–453.

Osella, A.R., Misciagna, G., Guerra, V., Elba, S., Buongiorno, G., Cavallini, A., Di Leo, A., Sonzogni, L. *et al.* (2001) Hepatitis C virus genotypes and risk of cirrhosis in southern Italy. *Clin Infect Dis* **33**, 70–75.

Pawlak, K., Pawlak, D. and Mysliwiec, M. (2004) Hepatitis intensified oxidative stress, MIP-1beta and RANTES plasma levels in uraemic patients. *Cytokine* **28**, 197–204.

Scully, C. and Greenspan, J.S. (2006) Human immunodeficiency virus (HIV) transmission in dentistry. *J Dent Res* **85**, 794–800.

Sezer, S., Tutal, E., Aldemir, D., Turkoglu, S., Demirel, O.U., Afsar, B., Ozdemir, F.N. and Haberal, M. (2006) Hepatitis C infection in hemodialysis patients: protective against oxidative stress? *Transplant Proc* **38**, 406–410.

Shepard, C.W., Finelli, L. and Alter, M.J. (2005) Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* **5**, 558–567.

Shukla, V., Kumar Shakya, A., Dhole, T.N. and Misra, U.K. (2012) Upregulated expression of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in BALB/c mouse brain challenged with Japanese encephalitis virus. *NeuroImmunoModulation* **19**, 241–254.

Sies, H. (1997) Oxidative stress: oxidants and antioxidants. *Exp Physiol* **82**, 291–295.

Tarantola, A., Abiteboul, D. and Rachline, A. (2006) Infection risks following accidental exposure to blood or body fluids

in health care workers: a review of pathogens transmitted in published cases. *Am J Infect Control* **34**, 367–375.

Tutal, E., Sezer, S., Ibis, A., Bilgic, A., Ozdemir, N., Aldemir, D. and Haberal, M. (2010) The influence of hepatitis C infection activity on oxidative stress markers and erythropoietin requirement in hemodialysis patients. *Transplant Proc* **42**, 1629–1636.

Vendemiale, G., Grattagliano, I., Portincasa, P., Serviddio, G., Palasciamo, G. and Altomare, E. (2001) Oxidative stress in symptom-free HCV carriers: relation with ALT flare-up. *Eur J Clin Invest* **31**, 54–63.

Walsh, K.M., Timms, P., Campbell, S., MacSween, R.N. and Morris, A.J. (1999) Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinases-1 and -2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C: comparison using ROC analysis. *Dig Dis Sci* **44**, 624–630.

Wang, X.L., Rainwater, D.L., VandeBerg, J.F., Mitchell, B.D. and Mahaney, M.C. (2001) Genetic contributions to plasma total antioxidant activity. *Arteioscler Thromb Vasc Biol* **21**, 1190–1195.

Yang, S.F., Hsieh, Y.S., Lin, C.L., Hsu, N.Y., Chiou, H.L., Chou, F.P. and Chu, S.C. (2005) Increased plasma levels of urokinase plasminogen activator and matrix metalloproteinase–9 in non-small cell lung cancer patients. *Clin Chim Acta* **354**, 91–99.