Chemistry Research Journal, 2018, 3(1):139-145

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Matrix Metaloprotinase-8 as Serum Biochemical Marker Accurately Predict Liver Fibrosis in Hepatitis C Patients

Faten Zahran Mohamed[1], Ibrahim El-Sayed M. El-Deen[2], Dina Mohamed Mohamed Ismail[2]

[1]Chemistry Department, Faculty of Science, Zagazig University, Egypt
[2]Chemistry Department, Faculty of Science, Port Said University, Egypt

Abstract Objective: Liver biopsy is the gold standard for assessing hepatitis C virus (HCV)-relatsed fibrosis, but it is invasive and prone to complications. Aim. Our aim was to study and validate the performance of MMP-8 as simple blood marker of liver fibrosis in HCV patients in addition to GSH and NO. Patients and methods: A total of 75 Egyptian patients with clinically and laboratory confirmed hepatitis C patients (HCV) and liver fibrosis were included in the present study. They were recruited from the Internal Medicine Department, EL Ahrar Hospital, Zagazig, Egypt that approved the present study. Result: NO values were significantly increased gradually according to the progression of fibrosis degree (F1, F2, F3 and F4) compared to healthy control individuals (F0). Also, GST activity was extremely significant elevated gradually with the degree of liver fibrosis till F4 compared to healthy control individuals (F0). Meanwhile, GST activity was reduced in F4 compared to other fibrotic degrees (F1, F2, and F3). On the other hand, MMP-8 activity was extremely significant elevated gradually with the degree of liver fibrosis compared to healthy control individuals (F0). Conclusion: using of matrix metalloproteinase-8 a simple biochemical marker which accurately predicts different stages of liver fibrosis in patients with chronic HCV infection, and may substantially reduce the necessity for liver biopsy.

Keywords Metaloprotinase-8, Biochemical Marker, Hepatitis C

Introduction

HCV and its long-term resultant consequences, is a major endemic medical health problem in Egypt [1]. In the Nile Delta and Upper Egypt, infection rates can be much higher at around 26% and 28%, respectively [2]. Chronic hepatitis C virus infection is important globally as a cause of liver- related morbidity and mortality with hepatic fibrosis, cirrhosis and hepatocellular carcinoma as the major clinical sequelae [3]. Liver fibrosis is a significant health problem, with a worldwide mortality attributable to cirrhosis and primary liver cancer of around 1.5 million deaths per year [4]. In patients with chronic viral hepatitis, precise definition of the hepatic fibrosis stage is the most important parameter to assess the risk of disease progression and to decide for an immediate and appropriate antiviral therapy. In these patients liver biopsy represents the gold standard for valuating the presence, type and stage of liver fibrosis [5]. This procedure, however, is invasive, stressful for patients, costly, and difficult to standardize [6, 7].

Collagenase-2 (MMP-8), a member of the MMP family which has recently emerged as a candidate to play a protective role during tumor progression. MMP-8 is mainly produced by neutrophils, and it has been implicated in a

variety of tissue remodeling processes associated with inflammatory conditions [8]. Oxidative stress is a state of imbalance between the production and dismutation, or detoxification, of reactive oxygen species (ROS) by cellular mechanisms that can significantly affect signal transduction, gene expression, and functional responses of involved cells or cause cell damage. Evidence of oxidative stress has been detected in almost all the clinical and experimental conditions of chronic liver diseases with different etiology and fibrosis progression rate, and oxidative stress has been proposed as a major pro-fibrogenic mechanism [9,10]. Cellular glutathione (GSH) and related enzymes such as glutathione peroxidase (GSH-Px), glutathione S-transferase (GST) and glutathione reductase (GR) are among the principal protective mechanisms against endogenous and exogenous toxic substances and free radicals-mediated damage in liver tissue as well as in other tissues [11,12]. On the one hand, NO is important in the resolution of some viral infections; on the other hand, it could cause or potentiate deleterious effects on the host. Thus, advances in our knowledge of the role of NO in immunomodulation and in the pathogenesis of viral diseases could contribute to the development of vaccines and therapeutic strategies [13,14].

Patients and Methods

A total of 75 Egyptian patients with clinically and laboratory confirmed chronic hepatitis C (CHC) and liver fibrosis were included in the present study. They were recruited from the Internal Medicine Department, EL Ahrar Hospital, Zagazig, Egypt that approved the present study. An informed consent was obtained from each individual participated in the present study and all were fully informed concerning the nature of the disease and the diagnostic procedures involved. The HCV infection and liver fibrosis were diagnosed based on biochemical, serologic and histological criteria. In addition to 15 samples from healthy volunteers (negative control) were used.

Liver biopsy

Needle liver biopsy specimens (n = 75) were taken from the patients and examined by a pathologist unaware of the laboratory results. Biopsies were processed for diagnostic purposes, fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4 μm thick and routinely stained with hematoxyline and eosin stain. Fibrosis was assessed according to the Metavir scoring system on a five-point scale (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis and F4 = cirrhosis). Activity grading by the Metavir system (based on the intensity of periportal and lobular necro-inflammation) was scored as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity and A3 = severe activity. The presence of stage F2, F3 or F4 was termed 'significant fibrosis', whereas the term 'advanced fibrosis' was reserved for stage F3 or F4. The presence of stage F4 was termed 'liver cirrhosis' [15].

Blood samples

Blood samples were collected from all healthy and patients by vein-puncture within 2 weeks of liver biopsy. Sera were separated from the blood samples and tested fresh for the tested biochemical marker matrix metalloproteinase-8 by the quantitative sandwich enzyme immunoassay technique according to Monaghan et al. [16], in addition to glutathione s-transferase activity by using Biodiagnostic kit method (Biodiagnostic company, Dokki, Giza, Egypt), according to the method of Habig et al. [17] and nitric oxide (NO) level was determined by using Biodiagnostic kit method (Biodiagnostic company, Dokki, Giza, Egypt), according to the method of Montgomery and Dymock [18].

Statistical Analysis

All statistical analyses were done by a statistical software package (Statistical Package for Social Sciences (SPSS 15.0) for Microsoft Windows, SPSS Inc.). Descriptive results were expressed as mean ± SD and range or number (percentage) of patients with a condition. Differences in continuous variables were assessed using Student's t-test or analysis of variance (ANOVA) and X^2 test for categorical variables [19].

Results

Diagnosis of samples

The present study involved 75 patients with clinically and laboratory confirmed chronic hepatitis C and liver fibrosis in addition to 15 negative controls (healthy volunteers). Positive patients with liver fibrosis were divided into different degrees according to METAVIR system as: 22 patients were categorized as F1 by (29%), 26 were F2 by (35%), 9 were F3 by (12%), and 18 patients were F4 by (24%).

The mean values of NO, GST and MMP-8 levels in healthy control individuals (F0) were found to be 17.91 ± 1.15 (µmol/l), 27.24 ± 3.2(U/L) and 85.59 ± 9.27 (pg/ml) respectively. NO values were significantly increased gradually according to the progression of fibrosis degree to be 26.16 ± 2.8, 55.41 ± 7.8, 93.30 ± 4.6, 93.34 ± 10.23 in F1, F2, F3, and F4 by 46.06%, 209.55%, 420.94% and 421.16%; respectively; ($p<0.001$), compared to healthy control individuals (F0). While, there was no significant difference between F3 and F4 stages, (Fig. 1). Also, GST activity was extremely significant elevated gradually with the degree of liver fibrosis till F4, to be 169.43 ± 20.69, 394.43 ± 51.95, 565.44 ± 47.90, and 224.88 ± 22.76 in F1, F2, F3, and F4 by 521.98%, 1347.98%, 1975.77%, and 725.55; respectively; ($p<0.001$), compared to healthy control individuals (F0). Meanwhile, GST activity was reduced in F4 compared to other fibrotic degrees (F1, F2, and F3),(Fig.2). On the other hand, MMP-8 activity was extremely significant elevated gradually with the degree of liver fibrosis, to be 279.38 ± 27.13, 454.75 ± 28.58, 544.55 ± 25.11 and 846.11 ± 130.3 in F1, F2, F3, and F4 by 226.44%, 431.31%, 536.23% and 885.56%; respectively; ($p<0.001$), compared to healthy control individuals (F0), illustrated in(Fig.3).

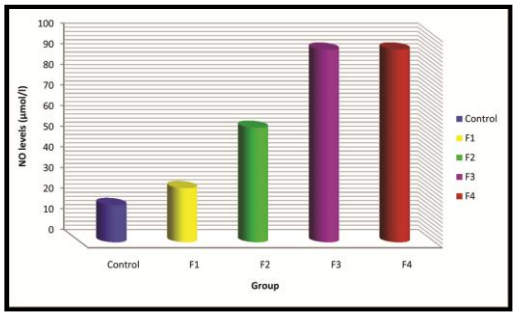

Figure 1: NO levels in all studied groups

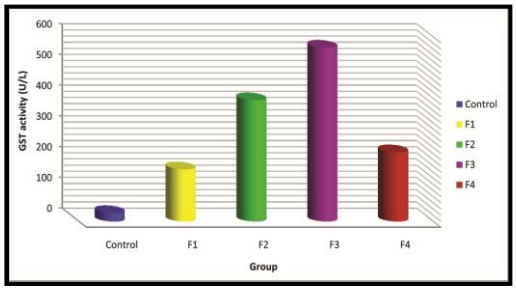

Figure 2: GST activity in all studied groups

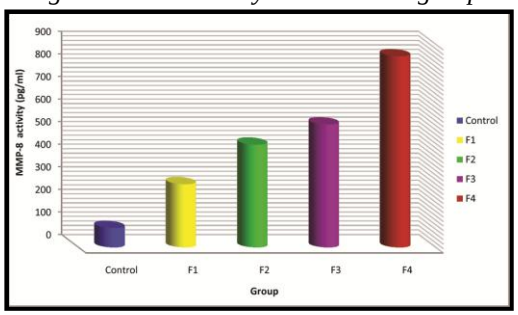

Figure 3: MMP-8 activity in all studied groups

Correlations between different studied parameters among studied groups:

There were significant positive correlations between NO, GST and MMP-8 to each other according to the degree of liver fibrosis (Table 1) and (fig. 4).

Table 1: Pearson's correlations analysis between different studied parameters in patients studied groups:

Parameter		NO	GST	MMP-8
NO	r	------	0.639**	0.898**
GST	r	0.639**	--------	0.465**
MMP-8	r	0.898**	0.465**	--------

**Correlation is significant at p<0.001

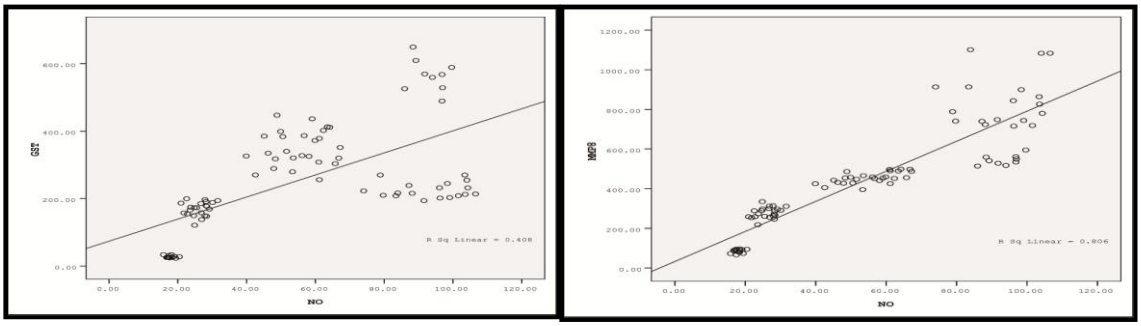

Figure 4: Correlations between different parameters in all studied groups

The laboratory data of liver fibrosis and non liver fibrosis, advanced liver fibrosis and liver cirrhosis

In the present study, patients with significant fibrosis were associated with higher mean NO, GST and MMP-8 than those of non significant liver fibrosis with extremely significant difference (p<0.0001; table 2). Patients with advanced liver fibrosis were associated with higher mean NO, GST and MMP-8 than those of non advanced liver fibrosis with extremely significant difference (p<0.0001; table 3). Also, patients with liver cirrhosis were associated with higher mean NO, GST and MMP-8 than those of non liver cirrhosis with extremely significant difference (p<0.0001; table 4).

Table 2: Levels of liver fibrosis markers in significant liver fibrosis and non significant liver fibrosis

Marker	Significant N=53	Non significant N=37	P value
NO(µmol/l)	56.9±35.5	17.9±1.2	< 0.0001
GST (U/l)	343.8±123.4	111.1±72.5	< 0.0001
MMP-8 (pg/ml)	529.3±287.8	85.1±9.3	< 0.0001

Table 3: Levels of liver fibrosis markers in advanced liver fibrosis and non advanced liver fibrosis

Marker	Advanced N=27	Non advanced N=63	P value
NO(µmol/l)	82.1 ±28.6	23.2± 13.9	< 0.0001
GST (U/l)	338.4±166.8	209.8 ±134.3	< 0.0001
MMP-8 (pg/ml)	745±179	175.8±162	< 0.0001

Table 4: Levels of liver cirrhosis markers in liver cirrhosis and non liver cirrhosis

Marker	cirrhosis N=18	Non cirrhosis N=72	P value
NO(µmol/l)	93.3± 10.2	27.2±22.1	< 0.0001
GST (U/l)	224.8± 22.7	254.3±173	< 0.0001
MMP-8 (pg/ml)	846.1±130	221.8± 195.4	< 0.0001

Discussion

Clinical management of chronic hepatitis C is dependent on the extent of liver fibrosis. Liver biopsy, the gold standard, is still recommended in the majority of patients [20]. However, liver biopsy is invasive, requires an experienced gastroenterologist, examination is required by a professional histopathologist, adds expense and is associated with complications and mortality patients with chronic hepatitis C [21,22]. Biomarkers are being developed as alternatives to liver biopsy for predicting liver fibrosis in patients with chronic hepatitis C [23].

In the present study, nitric oxide (NO), glutathione s-transferase (GST) and matrix metalloproteinase-8 (MMP-8) were measured using standard methodologies in 75 patients with clinically and laboratory confirmed chronic hepatitis C and liver fibrosis in addition to 15 negative controls (healthy volunteers).The mean NO, GST and MMP-8 levels in healthy control individuals (F0) were found to be 17.91 ± 1.15 (μmol/l), 27.24 ± 3.2(U/L) and 85.59 ± 9.27 (pg/ml) respectively. Nitric oxide (NO) values were significantly increased gradually according to the progression of fibrosis degree to be 26.16 ± 2.8, 55.41 ± 7.8, 93.30 ± 4.6, 93.34 ± 10.23 in F1, F2, F3, and F4 by 46.06%, 209.55%, 420.94% and 421.16%; respectively; ($p<0.001$), compared to healthy control individuals (F0). While, there was no significant difference between F3 and F4 stages. Hepatic fibrosis in patients chronically infected with hepatitis B and C also appears to be correlated with increased expression of iNOS. Although the molecular mechanisms have not been well elucidated, it was shown that fibrosis levels were correlated positively with iNOS expression, as well as that of TGF-b, which is an oxidative stress inducer and profibrogenic cytokine [24]. There is a controversy regarding the production of NO in chronic HCV patients with studies reporting an increase [25,26], a decrease [27], or no change [28,29] in its level.

During this study, Glutathione s-transferase (GST) activity was extremely significant elevated gradually with the degree of liver fibrosis till F4, to be 169.43 ± 20.69, 394.43 ± 51.95, 565.44 ± 47.90, and 224.88 ± 22.76 in F1, F2, F3, and F4 by 521.98%, 1347.98%, 1975.77%, and 725.55; respectively; ($p<0.001$), compared to healthy control individuals (F0). Meanwhile, GST activity was reduced in F4 compared to other fibrotic degrees (F1, F2, and F3). An increased GSH level in chronic liver diseases has been reported in many reports [30,31]. GST is a sensitive marker in the diagnosis of alcoholic liver disease [32] as well a reliable marker in monitoring the response to chronic liver disease treatment [33]. In both clinical and experimental HCC, reduced global activity of GST has been observed within tumors [11], and the specific isoforms GSTπ1 and GSTα1 [34,35] have been shown to be over expressed and have been used as biomarkers in experimental models of HCC.

In the current study, Matrix metalloproteinase-8 (MMP-8) activity was extremely significant elevated gradually with the degree of liver fibrosis, to be 279.38 ± 27.13, 454.75 ± 28.58, 544.55 ± 25.11 and 846.11 ± 130.3 in F1, F2, F3, and F4 by 226.44%, 431.31%, 536.23% and 885.56%; respectively; ($p<0.001$), compared to healthy control individuals (F0). MMP-8, a member of the MMP family which has recently emerged as a candidate to play a protective role during tumor progression. MMP-8 is mainly produced by neutrophils, and it has been implicated in a variety of tissue remodeling processes associated with inflammatory conditions [8]. The predominant role of matrix metalloproteinase 8 in extracellular matrix turnover, modulation of inflammatory responses and other physiological processes is well documented. Several recent studies highlight the involvement of MMP8 in a wide range of pathologies and as a disease marker in some inflammatory disorders and in cancer progression [36]. Under physiological condition, inactive MMP-8 is expressed at low levels in tissues and body fluids, but its level and activation increase significantly under various pathological conditions, e.g., inflammatory, fibrosis diseases and cancers [37,38].

From this study we conclude the following:

Matrix metalloproteinase-8 (MMP-8) can be used as a potential biomarker for the diagnosis of liver fibrosis. It was shown that fibrosis levels were correlated positively with Nitric oxide (NO) concentration.Glutathione s-transferase (GST) activity was a reliable monitoring the response to chronic liver disease treatment. Addition of (NO) and (GST) to (MMP-8) gives a significant improvement in detection of different stages of fibrosis in patients with HCV. Therefore, combination of multiple markers may be more valuable in the diagnosis of liver fibrosis. Finally, There were significant positive correlations between NO levels, activity of GST with MMP-8 to each other according to the degree of liver fibrosis.

We can recommended that, using of matrix metalloproteinase-8 a simple and non-invasive biochemical marker for the assessment of different stages of hepatic fibrosis as alternatives to liver biopsy which is invasive, expensive, painful and in some settings impossible to do in patients with chronic HCV infection in addition of glutathione s-transferase activity and level of nitric oxide.

References

1. El-Zanaty, F. and Way, A. Egypt Demographic and Health Survey 2008. Cairo, Egypt. Ministry of Health, El-Zanaty and Associates, and Macro International 2009; 431-439.
2. Elgharably, A., Gomaa, A.I., Crossey, M.M., Norsworthy, P.J., Waked, I. and Taylor-Robinson, S.D. Hepatitis C in Egypt – past, present, and future. Int. J. General Med. 2017; 10: 1–6.
3. Yarlott, L.; Heald, E. and Forton, D. Hepatitis C virus infection, and neurological and psychiatric disorders–A review. J. Adv. Res. 2017; 8: 139–148.
4. Fagone, P.; Mangano, K.; Pesce, A. and Portale, T.R. Emerging therapeutic targets for the treatment of hepatic fibrosis. Drug Disc. Tod. 2016; 21(2): 369-375.
5. Afdhal NH and Nunes D. Evaluation of liver fibrosis: a concise review. Am. J. Gastroenterol. 2004; 99: 1160-74.
6. Plebani M and Basso D. Non-invasive assessment of chronic liver and gastric diseases. J. Clinica. Chimica. Acta. 2007; 381: 39-49.
7. Sheiko, M.A. and Rosen, H.R. Hepatic Fibrosis in Hepatitis C. In: T. Miyamura et al. (eds.), Hepatitis C Virus II, Springer, Japan 2016; 79-108.
8. Gutierrez-Fernandez, A.; Fueyo, A.; Folgueras, A.R.; Garabaya, C., et al. Matrix Metalloproteinase-8 Functions as a Metastasis Suppressor through Modulation of Tumor Cell Adhesion and Invasion. Cancer Res. 2008; 68(8): 2755-2763.
9. Rebbani, K. and Tsukiyama-Kohara, K. HCV-Induced Oxidative Stress: Battlefield-Winning Strategy. Oxid. Med. Cell. Long. 2016; 7:1576-1589.
10. Camini, F.C.; Caetano, C.C.; Almeida, L.T. and Magalhaes, C.L. Implications of oxidative stress on viral patho-genesis. Arch. Virol. 2017; 162: 907– 917.
11. Czeczot H, Scibior D, Skrzycki M and Podsiad M. Glutathione and GSH- dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. Acta Biochim. Pol. 2006; 53(1): 237–242.
12. Sánchez-Rodríguez, R.; Torres-Mena, J.E.; Yauner, L.D. and Pérez-Carreón, J.I. Biomarkers of the Antioxidant Response: A Focus on Liver Carcinogenesis. In: V.R Preedy (ed.), Biomarkers in LiverDisease, Biomarkers in Disease: Methods, Discoveries and Applications. Springer Science+Business Media Dordrecht. 2016; 15: 1-24.
13. Nahrevanian, H. and Amini, M. Nitric Oxide Functions; an Emphasis on its Diversity in Infectious Diseases. Iran. J. Basic Med. Sci. 2009; 11(4): 197-204.
14. Uehara, E.U.; Shida, B.S. and de Brito, C.A. Role of nitric oxide in immune responses against viruses: beyond microbicidal activity. Inflamm. Res. 2015; 64: 845–852
15. Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterol. 1997; 122(5): 1303–1313.
16. Monaghan M., Browne S., Schenke-Layland K., and Pandit A. A Collagen-based Scaffold Delivering Exogenous Micro RNA-29B to Modulate Extracellular Matrix Remodeling. Mol. Ther. 2014; 22(4): 786-796.
17. Habig, W.H.; Pabst, M.J. and Jakoby, W.B. glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 1974; 249: 7130-7139.
18. Montgomery, H.C. and Dymock, J.F. The determination of nitrite in water. Analyst. 1961; 86: 414-416.
19. Levesque R. Programming and Data Management: A Guide for SPSS and SAS Users, Fourth Edition, SPSS Inc., Chicago, USA. 2007.

20. Strader DB, Wright T, Thomas DL, and Seeff LB. Diagnosis, management, and treatment of hepatitis C. J. Hepatol. 2004; 39:1147–71.
21. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR and Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am. J. Gastroenterol. 2002; 97: 2614-2618.
22. Bedossa P, Dargere D and Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. J. Hepatol. 2003; 38: 1449-57.
23. Becker L, Salameh W, Sferruzza A, Zhang K, ng Chen R, Malik R, Reitz R, Nasser I, and Afdhal NH. Validation of hepascore, compared with simple indices of fibrosis, in patients with chronic hepatitis C virus infection in United States. J. Clin. Gastroenterol. Hepatol. 2009; 7(6):696-701.
24. Tache DE, Stanciulescu CE, BaniTa IM, Purcaru SO, et al. Inducible nitric oxide synthase expression (iNOS) in chronic viral hepatitis and its correlation with liver fibrosis. Rom. J. Morphol. Embryol. 2014; 55:539–543.
25. Hassan MI, Kassim SK, Ali HS, Sayed el DA and Khalifa A. Evaluation of nitric oxide (NO) levels in hepatitis C virus (HCV) infection: relationship to schistosomiasis and liver cirrhosis among Egyptian patients. Dis. Markers 2002; 8: 137-142.
26. Pata C, Yazar A, Altintas E, et al. Serum levels of intercellular adhesion molecule-1 and nitric oxide in patients with chronic hepatitis related to hepatitis C virus: connection fibrosis. Hepatogastroenterol. 2003; 50: 794-797.
27. Lee CH, Choi YH, Yang SH, Lee CW, Ha SJ and Sung YC. Hepatitis C virus core protein inhibits interleukin 12 and nitric oxide production from activated macrophages. Virol. 2001; 279: 271-279.
28. Lake-Bakaar G, Sorbi D and Mazzoccoli V. Nitric oxide and chronic HCV and HIV infections. Dig. Dis. Sci. 2001; 46: 1072-1076.
29. Tavares FN, Goncalves PL, Porto SA, Pereira FE and Ribeiro-Rodrigues R. Nitric oxide levels are not changed in saliva of patients infected with hepatitis C virus. Rev. Soc. Bras. Med. Trop. 2005; 38: 453-455.
30. Loguercio C, De Girolamo V, Federico A, et al. Relationship of blood trace elements to liver damage, nutritional status, and oxidative stress in chronic nonalcoholic liver disease. Biol. Trace Elem. Res. 2001; 81: 245-254.
31. Czuczejko J, Zachara BA, Staubach-Topczewska E, Halota W, and Kedziora J. Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. Acta Biochim. Pol. 2003; 50: 1147-1154.
32. Singh M, Aggarwal HK and Aggarwal SK. Significance of The Glutathione-S Trasferase activity and total thiols status in chronic alcoholics. J. Clin. Diag. Res. 2012; 6: 31-33.
33. Federico A, Tuccillo C, Crafa E and Loguercio C. The significance of alpha-glutathione S trasferase deteremination in patients with chronic liver diseases. Minerva Gastroenterol. Dirtol. 1999; 45: 181-185.
34. Albrethsen J, Miller LM, Novikoff PM and Angeletti RH. Gel-based proteomics of liver cancer progression in rat. Biochim. Biophys. Acta. 2011; 1814(10):1367–1376.
35. Suzuki S, Pitchakarn P, Ogawa K, Naiki-Ito A, et al. Expression of glutathione peroxidase 2 is associated with not only early hepatocarcinogenesis but also late stage metastasis. Toxicol.2013; 311(3):115–123.
36. Dejonckheere, E.; Vandenbroucke, R.E. and Libert, C. Matrix metalloproteinase8 has a central role in inflammatory disorders and cancer progression. Cyt. Gro. Fact. Rev. 2011; 22: 73–81.
37. Duarte, S.; Baber, J.; Fujii, T. and Coito, A.J. Matrix met-alloproteinases in liver injury, repair and fibrosis. Matrix Biol. 2015; 46: 147–156.
38. Robert, S.; Gicquel, T.; Victoni, T., Valenc¸ S., et al. Involvement of matrix metalloproteinases (MMPs) and inflammasome pathway in molecular mechanisms of fibrosis. Biosci. Rep. 2016; 36: 360-371.