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***Observational Study***

**Matrix metalloproteases and their tissue inhibitors in non-alcoholic liver fibrosis of human immunodeficiency virus-infected patients**

Collazos J *et al.* MMPs and liver fibrosis in HIV

**Julio Collazos, Eulalia Valle-Garay, Tomás Suárez-Zarracina,Angel-HugoMontes, José A Cartón, Víctor Asensi**

**Julio Collazos,** Infectious Diseases, Hospital de Galdácano, 48960 Vizcaya, Spain.

**Eulalia Valle-Garay, Angel-Hugo Montes,** Biochemistry and Molecular Biology, Hospital Universitario Central de Asturias, Oviedo University School of Medicine, 33006 Oviedo, Spain

**Tomás Suárez-Zarracina, José A Cartón, Víctor Asensi**, Infectious Diseases, Hospital Universitario Central de Asturias, Oviedo University School of Medicine, 33006 Oviedo, Spain

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**Correspondence to**:**Dr. Julio Collazos,** Infectious Diseases Unit, Hospital de Galdácano, Bº Labeaga s/n, 48960 Galdácano, Vizcaya, Spain. [med003033@gmail.com](mailto:med003033@gmail.com)

**Telephone**: +34-94-6032860

**Fax**: +34-94-6032867

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**Abstract**

***AIM***

To investigate the relationships among diverse metalloproteases (MMPs) and their tissue inhibitors (TIMPs) and non-alcoholic liver fibrosis in human immunodeficiency virus (HIV)-infected patients.

***METHODS***

Single nucleotide polymorphisms (SNPs) in *MMPs,* *TNF-α* and *CCR5* genes, and serum levels of MMPs and TIMPs were determined in HIV-infected individuals with/out hepatitis C virus (HCV) coinfection. A total of 158 patients were included, 57 of whom were HCV-coinfected. All patients drank < 50 g ethanol/day. Diverse SNPs (*MMP-1- 1607 1G/2G*, *MMP-8 -799C/T*, *MMP-9 -1562 C/T*, *MMP-13 -77A/G*, *TNF-α -308 G/A*, *CCR5-Δ32*), and serum levels of MMPs (2, 3, 8, 9 and 10) and TIMPs (1, 2 and 4) were assessed. Liver fibrosis was determined by transient elastometry, although other non-invasive markers of fibrosis were also considered. Significant liver fibrosis (F ≥ 2) was defined by a transient elastometry value ≥ 7.1 kPa.

***RESULTS***

A total of 34 patients (21.5%) had liver fibrosis ≥ F2. MMP-2 and TIMP-2 serum levels were higher in patients with liver fibrosis ≥ F2 (*P* = 0.02 and *P* = 0.03, respectively) and correlated positively with transient elastometry values (*P* = 0.02 and *P* = 0.0009, respectively), whereas MMP-9 values were negatively correlated with transient elastometry measurements (*P* = 0.01). Multivariate analyses showed that high levels of MMP-2 (OR = 2.397; 95%CI: 1.191-4.827, *P* = 0.014) were independently associated with liver fibrosis ≥F2 in the patients as a whole. MMP-2 (OR = 7.179; 95%CI: 1.210-42.581, *P* = 0.03) and male gender (OR = 10.040; 1.621-62.11, *P* = 0.013) were also independent predictors of fibrosis ≥ F2 in the HCV-infected subgroup. Likewise, MMP-2, TIMP-2 and MMP-9 were independently associated with transient elastometry values and other non-invasive markers of liver fibrosis. None of the six SNPs evaluated had any significant association with liver fibrosis ≥ F2.

***CONCLUSION***

Certain MMPs and TIMPs, particularly MMP-2, seems to be associated with non-alcoholic liver fibrosis in HIV-infected patients with/out HCV coinfection.

**Key words:** Human immunodeficiency virus; Hepatitis C virus; Liver fibrosis; Transient elastometry; Non-invasive fibrosis markers; Metalloproteases; Their tissue inhibitors; Genetic polymorphisms

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**Core tip**: The role of matrix metalloproteases (MMPs) and their tissue inhibitors (TIMPs) in the development of liver fibrosis is uncertain. We determined some SNPs, as well as the serum levels of diverse MMPs and TIMPs, in non-alcoholic, human immunodeficiency virus-infected patients with/out HCV coinfection, to evaluate their possible relationship with liver fibrosis as assessed by transient elastometry. MMP-2 was independently associated with significant fibrosis. Likewise, MMP-2, TIMP-2 and MMP-9 were independent predictors of transient elastometry values and of other non-invasive tests of fibrosis. No SNP was significantly associated with liver fibrosis. Our findings support the value of these markers in the evaluation of fibrosis.

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**INTRODUCTION**

Liver fibrosis is characterized by a pathological accumulation of extracellular matrix (ECM), reflecting an imbalance between enhanced matrix synthesis and reduced breakdown of connective tissue proteins. ECM degradation is mediated by matrix metalloproteases (MMPs), a large family of zinc-dependent endopeptidases. Different levels of MMP regulation ensure the constant remodeling of the ECM, including regulation at the gene expression level, cleavage of the pro-enzyme to an active form, and specific inhibition of activated forms by tissue inhibitors (TIMPs)[1,2]. The relevance of MMPs for liver ECM remodeling is shown by the fact that pro-MMP-2 and pro-MMP-9 are activated during rat liver regeneration following hepatectomy and both MMPs contribute to priming hepatocyte proliferation[3]. Different genetic polymorphisms (SNPs) of MMPs and TIMPs have been described. Some of them such as the *MMP-1- 1607 1G/2G , MMP-3 -1612 5A/6A,* *MMP-9-1562 C/T* and *MMP-13 -77 A/G* are located in the MMPs genes promoter region and induce changes in MMPs genes mRNA and protein expression. These functional *MMPs* SNPs are associated mostly with cardiovascular diseases, but also with cancer and osteomyelitis susceptibility[4,5]. *MMP-1*, *MMP-3* and *MMP-9* SNPs have been associated with progression of liver disease in hepatitis C virus (HCV)-monoinfected Japanese patients[6]. Carriage of the *MMP-3 -1612 5A/6* SNP *6A* allele has been associated with increased albumin-globulin ratios in HCV-infected Mexican patients with advanced LF[7]. A *MMP14* SNP has also been associated with hepatocellular carcinoma[8].

Different studies have shown a correlation between TIMP-1, MMP-2 and MMP-9 serum levels and increased LF in HCV-monoinfected and human immunodeficiency virus (HIV)-HCV-coinfected individuals[9-13]. The Fibro-check, a combination of direct and indirect markers for LF stages in chronic hepatitis C, is constructed combining collagen III and its degrading enzyme MMP-1[14].

The aim of this study was to investigate the relationships among diverse *MMPs* SNPs, MMPs and TIMPs serum levels and non-alcoholic LF, evaluated by means of different non-invasive markers, in HIV-infected patients with and without HCV coinfection.

**MATERIALS AND METHODS**

***Study patients and data collection***

A total of 158 patients from the Infectious Diseases Outpatient Clinic of the Hospital Universitario Central de Asturias, a third-level 1500-bed University Hospital at Oviedo, Northwestern Spain, were included in the study. Patients older than 18 years with active human immunodeficiency virus (HIV) or HIV-HCV coinfection, demonstrated by positive serology and viral RNA plasma detection, were enrolled. Demographic, analytical and clinical data, including ethanol and drug consumption, were obtained from patients and their medical charts at enrollment. In addition, we did transient elastometry (TE) to determine the degree of LF. Patients with alcohol abuse, defined as an ethanol intake of ≥ 50 g/d for > 5 years, were excluded. Many patients were not aware of how long they were HCV infected. In such cases, it was assumed that the patients acquired HCV-infection one year after starting intravenous drugs, as previously reported[15]. All patients were receiving antiretroviral therapy (ART) at the time of inclusion. All patients underwent standard care, including routine non-invasive procedures. Patients were members of a homogeneous Caucasian population, and were residents in the same region (Asturias, Northern Spain) that has a small foreign immigrant population (less than 5%).

***Exclusion criteria***

Pregnant women and those individuals in whom there were technical difficulties for obtaining reliable TE readings were excluded from the study. In addition, patients with an acute episode of cytolysis or cholestasis, ascitis or spontaneous bacterial peritonitis were excluded because TE reading could be altered by these factors[15]. We also excluded patients who currently or previously were treated with anti-HCV therapy and those who had resolved their HCV infections spontaneously (defined as positive serology but with undetectable HCV RNA). Patients with ART adherence < 75% were also excluded. To avoid other confounding factors, patients with HBV coinfection with/out delta virus coinfection, ethanol consumption ≥ 50 g/d for > 5 years, alcoholic hepatopathy, other liver diseases, or treatment with immunosuppressant drugs, were excluded from the study as well.

***Laboratory methods***

HIV and HCV serologies were determined by enzyme immunoassay (MEIA AxSYM; Abbott Diagnostics, Abbott Park, IL, United States). HIV and HCV RNA by quantitative PCR (Cobas TaqMan; Roche Diagnostics, Branchburg, NJ, United States) and HCV genotypes by a line probe assay (Versant HCV, Siemens). Routine laboratory methods were used to calculate LF indexes: AST and platelets for APRI index[16], age, platelet counts, total cholesterol and GGT for calculating the Forns index[17], and age, AST, ALT and platelet counts for FIB-4[18]. In addition, the Yearly Fibrosis Progression Index (YFPI) was also calculated in HCV-infected patients as follows: YFPI = TE value/years of estimated HCV infection.

***Transient elastometry***

LF was assessed by TE using Fibroscan (EchoSens, Paris, France) following pre-established methods[15,19]. Patients were divided into four groups according to TE measurements, reflecting the progressive stage of LF and analogous to the F0–1, F2, F3 and F4 histological stages of the Metavir scoring system. The TE cut-offs used for this purpose were those described by Castera *et al*[20]: F0-1: < 7.1 kPa, F = 2: 7.1-9.4 kPa, F = 3: 9.5-12.4 kPa and F = 4 ≥ 12.5 kPa.

***MMPs and TIMPs serum levels assessment***

Ten mL of whole blood were drawn in siliconized glass tubes, and centrifuged at 1800 × g for 5 min. The obtained serum was aliquoted in Eppendorf tubes and stored at -70 ºC until further use. MMPs (-2, -3, -8, -9, -10) and TIMPs (-1,-2,-4) were measured by the QuantibodyTM Human MMP Array 1 (RayBiotech, Parkway Lane, Norcross, GA, USA), according to the manufacturer’s instructions and as previously published by our group[21].

***MMPs SNPs genotyping***

DNA was obtained from peripheral white blood cells and stored at -20ºC before use. The following SNPs of MMPs were genotyped by PCR: *MMP-1 (-1607 1G/2G,* rs 11292517), MMP-8 *(-799 C/T*, rs 11225395), *MMP-9 (-1562 C/T,* rs 34016235), and *MMP-13 (-77 A/G,* rs 2252070). In addition the *TNF-α* (*-308 G/A*, rs 1800629) and the *CCR5 Δ32* (rs 333) SNPs were also genotyped. Oligonucleotide primer sequences, PCR conditions and restriction enzymes used for genotyping and sequencing of the different SNPs studied have been described elsewhere[5,21-23].

***Statistical analysis***

As MMPs and TIMPs serum levels presented a markedly non-Gaussian distribution, original values were logarithmically transformed for analysis. The reported values are the result of back-transformation into the original units (ng/mL). Continuous variables are presented as mean (95%CI). Proportions were compared with the chi-square test, whereas t-test and one-way analysis of variance were used for the comparison of continuous variables in two or more than two groups, respectively. Correlations between MMPs, TIMPs and LF indexes were assessed with the Pearson’s correlation coefficient. Stepwise logistic regression analyses were carried out to find the factors independently associated with significant LF, and stepwise multiple regressions were performed to detect the parameters independently predictive of the different LF indexes. SPSS v.22 software was used for statistical calculations. A *P* value < 0.05 for a two-tailed test was considered statistically significant.

**RESULTS**

The study population was composed of 158 HIV-infected patients, 57 (36.1%) of whom were coinfected with HCV. The mean age was 44.6 years, 65.8% were male, the mean CD4 counts were 581.5 cells/μL and 85.4% of them had undetectable HIV viral load. Thirty-four patients (21.5%) had significant LF (≥ F2).

Table 1 shows the demographic, clinical and laboratory data of the patients with and without LF ≥ F2, as well as the comparison between the two groups. As expected, HCV infection and IDU were associated with LF, but the estimated duration of HCV infection was not. Regarding the HIV-related parameters, both nadir and current CD4 counts were lower, and the duration of HIV infection and time on ART higher in patients with LF ≥ F2 than in patients without LF. There were no statistically significant differences in HIV or HCV viral loads between the two groups, although there was a trend towards higher HCV viral load and lower rates of undetectable HIV viral load in the patients with LF. The different HCV genotypes were similarly represented in the two groups and there were no significant associations between TE values and HCV genotypes (*P* = 0.5).

Not surprisingly, the laboratory parameters used for the calculations of the LF indexes, such as platelet count, cholesterol, AST, and GGT, differed significantly between the LF groups. Regarding MMPs and TIMPs, MMP-2 and TIMP-2 serum levels were significantly higher in LF than in patients without LF.

Table 2 shows the genotypic frequencies of the SNPs evaluated according to LF and HCV status. No genotype or SNPs was significantly associated with any of the two conditions.

The relationships of the SNPs and the LF markers are detailed in Table 3. No statistically significant association was found between the different SNPs and the LF indexes, including TE, although patients carrying the heterozygous *CT* genotype of the *MMP-9 -1562 C/T* SNP had consistently higher values of all LF indexes than those with the homozygous *CC* genotype. Table 4 shows the comparisons of the MMPs and TIMPs serum levels according to the different SNPs. Statistically significant differences were observed only between *MMP-8 -799C/T* SNP and TIMP-2 (*P* = 0.01), *MMP-9-77A/G* SNP and MMP-2 (*P* = 0.02) and *TNF-α -308 G/A* SNP and TIMP-4 levels (*P* = 0.02).

Table 5 summarizes the correlations between the different MMPs, TIMPs and LF indexes. There was a good positive correlation among the different LF indexes (*P* < 0.0001 for all comparisons). Likewise, the diverse fibrosis indexes correlated positively with MMP-2 (*P* = 0.02 to *P* = 0.06) and TIMP-2 (*P* = 0.08 to *P* < 0.0001) and negatively with MMP-9 (*P* =0.2 to *P* = 0.01). Also, the different MMPs and TIMPs correlated among them. There were strong correlations between MMP-8 levels and levels of MMP-9 and TIMP-1, and between MMP-9 and TIMP-1 levels (*P* < 0.0001 for each comparison), which explained about a half of the variability of their values.

***Multivariate analyses***

The variables with a *P* ≤ 0.2 significance level in the univariate analyses were entered into the different multivariate models for LF evaluation, excluding the parameters directly indicative of LF and the laboratory tests used for their calculations.

Stepwise logistic regression analyses revealed that high serum levels of MMP-2 (OR = 7.179; 1.210-42.581, *P* = 0.03) and male gender (OR = 10.040; 1.621-62.11, *P* = 0.013) were independent predictors of fibrosis ≥F2 in the HCV-infected subgroup. In the patients as a whole, only MMP-2 (OR = 2.397, 95%CI: 1.191-4.827, *P* = 0.014) was independently associated with LF ≥F2, whereas gender was close to the significance level (*P* = 0.08).

Multiple regression analyses were also carried out to evaluate the factors independently associated with each of the five LF indexes (TE, APRI, Forns, FIB-4, and YFPI). Among the different variables considered, only five factors (MMP-2, TIMP-2, MMP-9, CD4 counts and age) explained the diverse markers evaluated. MMP-2 was the parameter most consistently predictive of these indexes. Table 6 shows the p values corresponding to these associations, as well as the adjusted percentage of variability of each LF index accounted for by the model.

The SNPs we evaluated did not have any significant association in the multivariate analyses with either LF ≥ F2 or any of the different LF indexes analyzed.

**DISCUSSION**

We found that serum MMP-2 was an independent predictor of non-alcoholic LF ≥ F2 in HIV-infected patients, as evaluated by TE. In addition, higher serum levels of MMP-2 and TIMP-2, as well as lower levels of MMP-9, were also predictive of higher scores of the diverse laboratory-derived indexes commonly used to measure the degree of LF. Taking into account that these LF indexes are calculated by means of different parameters, the consistent association of these MMPs and TIMPs with each of them reinforces our findings and the value of these MMPs and TIMPs as additional markers of LF. Our results agree with those of Macias *et al*[13] that found an association of serum MMP-2 with LF measured by liver biopsy in 90 HIV-HCV-coinfected Spanish patients. These authors suggested that the combination of AST, platelet count and serum MMP-2 levels is a biochemical surrogate marker for LF ≥ F2.

We did not observe any association between serum TIMP-1 and LF, or any of the multiple fibrosis indexes studied, as was reported by others studying heterogeneous aspects related to fibrosis in HCV-monoinfected or HIV-HCV-coinfected individuals[9-12]. On the contrary, we found an independent association of serum MMP-2, MMP-9 and TIMP-2 with diverse LF indexes. We did not measured serum MMP-1, which was associated with LF in HCV-monoinfected individuals in another study and was included in the Fibro-check[14].

We did not find any statistically significant association between LF and the different SNPs evaluated, although patients carrying the heterozygous *CT* genotype of the *MMP-9 -1562 C/T* SNPhad consistently higher values of all LF indexes than those with the homozygous *CC* genotype. Okamoto *et al* reported an association of *MMP-1- 1607 1G/2G*, *MMP-3 -1612 5A/6* and *MMP-9 -1562 C/T*, SNPs with LF progression measured by biochemical markers or liver biopsy in HCV-monoinfected Japanese patients[6]. Sánchez-Parada *et al*[7] found that *TGBFB1* +915 C/G (rs 1800471) SNP carriage was associated with severity of hepatic necroinflammation and LF in HCV-monoinfected Mexican patients. In addition, the same authors reported an association between *MMP-3 -1612 5A/6* SNP *6A* allele carriage and an increase in the albumin-globulin ratio, as a surrogate marker of LF. The ethnic background of our patients was different from those of previous reports, and the relatively small sample size of our HIV-HCV-coinfected population could perhaps explain these discrepant findings. We did not genotype the *TGBFB1* +915 C/G nor the *MMP14* (-1658 [rs100349], +7096 [rs2236307] and + 8153 [rs3751489]) SNPs that have been associated with LF and hepatocellular carcinoma in HCV-monoinfected patients of Mexican and Chinese extraction[7,8].

We found that male gender was independently associated with LF in HIV-HCV coinfection. This association was already described by our group in another cohort of patients[24], and appears to be at least partially due to hormonal issues. In this regard, experimental studies in rats have shown the beneficial effects of estradiol administration on LF through diverse mechanisms[25-28].

Limitations to our study include those inherent to cross-sectional studies and the relatively small number of patients with LF ≥ F2. The relatively small sample size might affect especially the genetic testing results. However, the sample size was large enough to find significant associations between LF and MMPs, TIMPs and other factors, and our findings on the independent relationships of MMPs and TIMPs with the diverse LF markers evaluated were highly consistent and support the reliability of our results.

A possible reason for the small numbers of patients with LF ≥2 is the exclusion of alcoholics from this study. We used a definition of alcohol abuse based on an ethanol exposition ≥50 g/d for >5 years previously used by others and us[29,30]. This alcohol consumption equates to approximately 3.5 drinks per day using standard drinks in the United States. Other authors reduced the alcohol abuse to ≥ 40 g/d for > 5 years[12]. We consider that this discrepancy might play a minor role in our study considering that only 34 patients (21.5% of the total) had LF ≥ F2 and 32 of them had HIV-HCV coinfection.

We conclude that some MMPs and TIMPs, such as MMP-9, TIMP-2 and especially MMP-2, are associated with non-alcoholic LF and diverse fibrosis markers in HIV-infected patients with and without HCV coinfection. The determination of these parameters could be useful for the development of other laboratory-derived indexes of LF in order to improve the accuracy of the current non-invasive tests. On the contrary, the SNPs evaluated did not significantly associate with LF in our Caucasian cohort, although this aspect needs to be confirmed by other studies with larger sample sizes and, perhaps, with patients of different ethnic extraction, taking into account the trend that we observed with the *MMP-9 1562* SNP.

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**COMMENTS**

***Background***

Liver fibrosis reflects an imbalance between extracellular matrix synthesis and reduced breakdown of connective tissue proteins, which is regulated by matrix metalloproteases (MMPs) and their tissue inhibitors (TIMPs). Genetic polymorphisms (SNPs) of MMPs and TIMPs induce changes in *MMPs* genes mRNA and protein expression. However, the role of MMPs, TIMPs and their SNPs in the development of liver fibrosis and their usefulness for the evaluation of fibrosis in clinical practice are uncertain.

***Research frontiers***

Some studies have inconsistently found a relationship between liver fibrosis and certain MMPs, TIMPs and SNPs in hepatitis C virus (HCV)-monoinfected and human immunodeficiency virus (HIV)-HCV-coinfected individuals, although the issue is far from clear. The topic is important, not only to support a possible pathogenic role of these substances and their genetic polymorphisms in the generation of fibrosis, but also to define the possible value of these determinations in the evaluation of the degree of fibrosis, which could be useful to clinicians involved in the care of these patients.

***Innovations and breakthroughs***

Excessive alcohol intake, a common habit among intravenous drug users, most of whom are also coinfected with HCV, is a cause of liver disease, and the influence of MMPs, TIMPs and their SNPs might vary according to the etiology of liver fibrosis. Consequently, we excluded patients with excessive alcohol intake, to minimize the possible confounding factor of multiple etiologies of fibrosis. On the other hand, non-invasive methods of measurement of liver fibrosis, mainly transient elastometry, are replacing liver biopsy in the evaluation of the degree of fibrosis. Therefore, the authors have also analyzed the relationships of these substances with multiple fibrosis indexes, in order to verify the consistence of such relationships from the perspective of different fibrosis markers. The authors found that high levels of MMP-2 were independently associated with liver fibrosis ≥ F2. Likewise, MMP-2, TIMP-2 and MMP-9 were independent and consistent predictors of transient elastometry values and of other non-invasive markers of fibrosis. On the contrary, we did not find any significant association between liver fibrosis ≥F2 and the diverse SNPs evaluated.

***Applications***

This study supports the implication of these substances in the development of liver fibrosis, and their value as predictors of the degree of fibrosis in HIV-infected patients with non-alcoholic liver disease. The determination of these parameters could be useful for the development of laboratory-derived indexes of fibrosis, in order to improve the accuracy of the current non-invasive tests.

***Terminology***

MMPs, a family of zinc-dependent endoproteases, and their tissue inhibitors TIMPs are involved in the remodeling and degradation of the extracellular matrix and, therefore, may influence the development of liver fibrosis.

***Peer-review***

The results presented in this reviewed manuscript are of scientific merit and interest.**REFERENCES**

1 **Parks WC**, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 2004; **4**: 617-629 [PMID: 15286728 DOI: 10.1038/nri1418]

2 **Mastroianni CM**, Lichtner M, Mascia C, Zuccalà P, Vullo V. Molecular mechanisms of liver fibrosis in HIV/HCV coinfection. *Int J Mol Sci* 2014; **15**: 9184-9208 [PMID: 24865485 DOI: 10.3390/ijms15069184]

3 **Kim TH**, Mars WM, Stolz DB, Michalopoulos GK. Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. *Hepatology* 2000; **31**: 75-82 [PMID: 10613731 DOI: 10.1002/hep.510310114]

4 **Ye S**. Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. *Cardiovasc Res* 2006; **69**: 636-645 [PMID: 16122719 DOI: 10.1016/j.cardiores.2005.07.015]

5 **Montes AH**, Valle-Garay E, Alvarez V, Pevida M, García Pérez E, Paz J, Meana A, Asensi V. A functional polymorphism in MMP1 could influence osteomyelitis development. *J Bone Miner Res* 2010; **25**: 912-919 [PMID: 19821768 DOI: 10.1359/jbmr.091013].]

6 **Okamoto K**, Mimura K, Murawaki Y, Yuasa I. 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* 2005; **20**: 1102-1108 [PMID: 15955221 DOI: 10.1111/j.1440-1746.2005.03860.x]

7 **Sánchez-Parada MG**, Alvarez-Rodríguez BA, Gómez-Meda BC, Troyo-Sanromán R, Sánchez-Orozco LV, Zamora-Perez AL, Landeros MS, Armendáriz-Borunda J. Association of genetic polymorphisms with histological grading of necroinflammation, staging of fibrosis, and liver function in Mexicans with chronic hepatitis C virus infection. *J Investig Med* 2013; **61**: 1088-1096 [PMID: 23941979 DOI: 10.2310/JIM.0b013e3182a32e24]

8 **Chen TY**, Li YC, Liu YF, Tsai CM, Hsieh YH, Lin CW, Yang SF, Weng CJ. Role of MMP14 gene polymorphisms in susceptibility and pathological development to hepatocellular carcinoma. *Ann Surg Oncol* 2011; **18**: 2348-2356 [PMID: 21298348 DOI: 10.1245/s10434-011-1574-x]

9 **Lichtinghagen R**, Huegel O, Seifert T, Haberkorn CI, Michels D, Flemming P, Bahr M, Boeker KH. Expression of matrix metalloproteinase-2 and -9 and their inhibitors in peripheral blood cells of patients with chronic hepatitis C. *Clin Chem* 2000; **46**: 183-192 [PMID: 10657374]

10 **Patel K**, Gordon SC, Jacobson I, Hézode C, Oh E, Smith KM, Pawlotsky JM, McHutchison JG. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; **41**: 935-942 [PMID: 15582126 DOI: 10.1016/j.jhep.2004.08.008]

11 **Fontana RJ**, Dienstag JL, Bonkovsky HL, Sterling RK, Naishadham D, Goodman ZD, Lok AS, Wright EC, Su GL. Serum fibrosis markers are associated with liver disease progression in non-responder patients with chronic hepatitis C. *Gut* 2010; **59**: 1401-1409 [PMID: 20675691 DOI: 10.1136/gut.2010.207423]

12 **Larrousse M**, Laguno M, Segarra M, De Lazzari E, Martinez E, Blanco JL, León A, Deulofeu R, Miquel R, Milinkovic A, Lonca M, Miró JM, Biglia A, Murillas J, Gatell JM, Mallolas J. Noninvasive diagnosis of hepatic fibrosis in HIV/HCV-coinfected patients. *J Acquir Immune Defic Syndr* 2007; **46**: 304-311 [PMID: 18172937 DOI: 10.1097/QAI.0b013e3181520502]

13 **Macías J**, Mira J, Gilabert I, Neukam K, Roldán C, Viloria M, Moro A, Pineda JA. Combined use of aspartate aminotransferase, platelet count and matrix metalloproteinase 2 measurements to predict liver fibrosis in HIV/hepatitis C virus-coinfected patients. *HIV Med* 2011; **12**: 14-21 [PMID: 20497249 DOI: 10.1111/j.1468-1293.2010.00836.x]

14 **Attallah AM**, El-Far M, Abdel Malak CA, Omran MM, Farid K, Hussien MA, Albannan MS, Attallah AA, Elbendary MS, Elbesh DA, Elmenier NA, Abdallah MO. Fibro-check: a combination of direct and indirect markers for liver fibrosis staging in chronic hepatitis C patients. *Ann Hepatol* 2015; **14**: 225-233 [PMID: 25671832]

15 **Cartón JA**, Collazos J, de la Fuente B, García-Alcalde ML, Suarez-Zarracina T, Rodríguez-Guardado A, Asensi V. Factors associated with liver fibrosis in intravenous drug users coinfected with HIV and HCV. *Antivir Ther* 2011; **16**: 27-35 [PMID: 21311106 DOI: 10.3851/IMP1708]

16 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

17 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992 [PMID: 12297848 DOI: 10.1053/jhep.2002.36128]

18 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]

19 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338 DOI: 10.1016/j.ultrasmedbio.2003.07.001]

20 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]

21 **Martin G**, Asensi V, Montes AH, Collazos J, Alvarez V, Carton JA, Taboada F, Valle-Garay E. Role of plasma matrix-metalloproteases (MMPs) and their polymorphisms (SNPs) in sepsis development and outcome in ICU patients. *Sci Rep* 2014; **4**: 5002 [PMID: 24833564 DOI: 10.1038/srep05002]

22 **Asensi V**, Rego C, Montes AH, Collazos J, Carton JA, Castro MG, Alvarez V, Fernández C, Maradona JA, Valle-Garay E. IL-1beta (+3954C/T) polymorphism could protect human immunodeficiency virus (HIV)-infected patients on highly active antiretroviral treatment (HAART) against lipodystrophic syndrome. *Genet Med* 2008; **10**: 215-223 [PMID: 18344712 DOI: 10.1097/GIM.0b013e3181632713]

23 **Alvarez V**, López-Larrea C, Coto E. Mutational analysis of the CCR5 and CXCR4 genes (HIV-1 co-receptors) in resistance to HIV-1 infection and AIDS development among intravenous drug users. *Hum Genet* 1998; **102**: 483-486 [PMID: 9600249]

24 **Collazos J**, Cartón JA, Asensi V. Gender differences in liver fibrosis and hepatitis C virus-related parameters in patients coinfected with human immunodeficiency virus. *Curr HIV Res* 2011; **9**: 339-345 [PMID: 21827383 DOI: 10.2174/157016211797635982]

25 **Yasuda M**, Shimizu I, Shiba M, Ito S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology* 1999; **29**: 719-727 [PMID: 10051473 DOI: 10.1002/hep.510290307]

26 **Xu JW**, Gong J, Chang XM, Luo JY, Dong L, Hao ZM, Jia A, Xu GP. Estrogen reduces CCL4- induced liver fibrosis in rats. *World J Gastroenterol* 2002; **8**: 883-887 [PMID: 12378635 DOI: 10.3748/wjg.v8.i5.883]

27 **Xu JW**, Gong J, Chang XM, Luo JY, Dong L, Jia A, Xu GP. Effects of estradiol on liver estrogen receptor-alpha and its mRNA expression in hepatic fibrosis in rats. *World J Gastroenterol* 2004; **10**: 250-254 [PMID: 14716833 DOI: 10.3748/wjg.v10.i2.250]

28 **Liu QH**, Li DG, Huang X, Zong CH, Xu QF, Lu HM. Suppressive effects of 17beta-estradiol on hepatic fibrosis in CCl4-induced rat model. *World J Gastroenterol* 2004; **10**: 1315-1320 [PMID: 15112349 DOI: 10.3748/wjg.v10.i9.1315]

29 **Merchante N**, Pérez-Camacho I, Mira JA, Rivero A, Macías J, Camacho A, Gómez-Mateos J, García-Lázaro M, Torre-Cisneros J, Pineda JA. Prevalence and risk factors for abnormal liver stiffness in HIV-infected patients without viral hepatitis coinfection: role of didanosine. *Antivir Ther* 2010; **15**: 753-763 [PMID: 20710057 DOI: 10.3851/IMP1612]

30 **Suárez-Zarracina T**, Valle-Garay E, Collazos J, Montes AH, Cárcaba V, Carton JA, Asensi V. Didanosine (ddI) associates with increased liver fibrosis in adult HIV-HCV coinfected patients. *J Viral Hepat* 2012; **19**: 685-693 [PMID: 22967099 DOI: 10.1111/j.1365-2893.2012.01596.x]

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**Table 1 Demographic, clinical and laboratory characteristics of the patients, according to the existence or not of significant liver fibrosis (≥ F2)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **All patients**  **(*n* = 158)** | **No fibrosis**  **(*n* = 124)** | **Fibrosis**  **(*n* = 34)** | ***P* value** |
| **Demography, epidemiology,**  **anthropometry and habits** |  |  |  |  |
| Age (yr) | 44.56 (43.20-45.92) | 44.50 (42.93-46.07) | 44.79 (41.96-47.63) | 0.9 |
| Male, *n* (%) | 104 (65.8) | 78 (62.9) | 26 (76.5) | 0.14 |
| Weight (kg) | 65.17 (62.88-67.46) | 64.46 (61.72-67.20) | 67.76 (64.03-71.50) | 0.24 |
| Height (cm) | 164.4(160.6-168.2) | 163.4 (158.5-168.2) | 168.2 (166.2-170.2) | 0.3 |
| Body mass index (kg/m2) | 23.58 (23.10-24.05) | 23.48 (22.95-24.01) | 23.90 (22.75-25.0.6) | 0.5 |
| Tobacco smokers, *n* (%) | 105 (66.5) | 78 (62.9) | 27 (79.4) | 0.07 |
| Cannabis use, *n* (%) | 38 (24.1) | 22 (17.7) | 16 (47.1) | 0.0004 |
| Alcohol intake, *n* (%)1 | 55 (35.0) | 43 (34.7) | 12 (36.4) | 0.9 |
| IDU, *n* (%) | 58 (36.9) | 29 (23.6) | 29 (85.6) | < 0.0001 |
| Men who have sex with men, *n* (%) | 28 (17.8) | 27 (22.0) | 1 (2.9) | 0.01 |
| Heterosexual, *n* (%) | 67 (42.7) | 63 (51.2) | 4 (11.8) | < 0.0001 |
| Transfusion, *n* (%) | 4 (2.5) | 4 (3.3) | 0 (0.0) | 0.3 |
| **HIV-related parameters** |  |  |  |  |
| Current CD4 counts (cells/μL) | 581.5 (533.0-630.1) | 612.3 (555.8-668.8) | 469.6 (383.7-555.4) | 0.017 |
| Nadir CD4 counts (cells/μL) | 201.8 (176.5-227.1) | 213.4 (183.4-243.4) | 159.7 (116.7-202.6) | 0.04 |
| CD4 gain (cells/μL) | 379.7 (334.4-425.0) | 399.0 (345.4-452.6) | 309.9 (231.4-388.4) | 0.11 |
| Undetectable HIV viral load, *n* (%) | 135 (85.4) | 109 (87.9) | 26 (76.5) | 0.09 |
| HIV viral load (log copies/mL)2 | 2.996 (2.556-3.434) | 2.991 (2.407-3.575) | 3.006 (2.173-3.840) | 0.97 |
| Years of HIV infection | 11.93 (11.12-12.73) | 11.31 (10.39-12.22) | 14.19 (12.70-15.68) | 0.003 |
| Months on antiretroviral therapy | 114.3 (106.8-121.8) | 109.9 (101.3-118.5) | 130.2 (115.0-145.3) | 0.03 |
| CDC clinical stage, *n* (%) |  |  |  |  |
| A | 84 (53.5) | 65 (52.8) | 19 (55.9) | 0.07 |
| B | 23 (14.6) | 22 (17.9) | 1 (2.9) |  |
| C | 50 (31.8) | 36 (29.3) | 14 (41.2) |  |
| **HCV-related parameters** |  |  |  |  |
| HCV infection, *n* (%) | 57 (36.1) | 25 (20.2) | 32 (94.1) | <0.0001 |
| HCV viral load (log copies/mL) | 5.745 (5.504-5.985) | 5.524 (5.085-5.964) | 5.915 (5.649-6.181) | 0.11 |
| Years of HCV infection | 22.46 (20.71-24.21) | 21.84 (18.96-24.72) | 22.94 (20.65-25.23) | 0.5 |
| HCV genotype, *n* (%) |  |  |  |  |
| 1 | 31 (54.4) | 14 (56.0) | 17 (53.1) | 0.7 |
| 2 | 2 (1.3) | 1 (4.0) | 1 (3.1) |  |
| 3 | 15 (23.6) | 5 (20.0) | 10 (31.3) |  |
| 4 | 9 (15.8) | 5 (20.0) | 4 (12.5) |  |
| **Liver fibrosis parameters** |  |  |  |  |
| Transient elastometry (kPa) | 7.53 (5.99-9.06) | 4.65 (4.45-4.85) | 18.02 (11.93-24.10) | < 0.0001 |
| APRI | 0.633 (0.511-0.756) | 0.385 (0.346-0.423) | 1.541 (1.093-1.989) | < 0.0001 |
| Forns | 4.412 (4.116-4.707) | 3.990 (3.734-4.246) | 5.949 (5.095-6.802) | 0.0001 |
| FIB-4 | 1.475 (1.249-1.700) | 1.088 (0.985-1.191) | 2.884 (2.031-3.736) | 0.0002 |
| YFPI 3 | 0.584 (0.416-0.752) | 0.281 (0.222-0.340) | 0.821 (0.548-1.095) | 0.0004 |
| Degree of liver fibrosis, *n* (%) |  |  |  |  |
| F0-F1 | 124 (78.5) | 124 (100) | 0 (0.0) | < 0.0001 |
| F2 | 15 (9.5) | 0 (0.0) | 15 (44.1) |  |
| F3 | 10 (6.3) | 0 (0.0) | 10 (29.4) |  |
| F4 | 9 (5.7) | 0 (0.0) | 9 (26.5) |  |
| **Laboratory parameters** |  |  |  |  |
| Platelet count (/μL) | 222570 (211690-233450) | 236750 (225410-248090) | 169270 (147220-191330) | < 0.0001 |
| Glucose (mg/dL) | 98.54 (95.25-101.84) | 98.00 (94.62-101.38) | 100.66 (90.92-110.39) | 0.5 |
| Total cholesterol (mg/dL) | 195.01 (188.51-201.51) | 201.0 (193.8-208.3) | 173.0 (160.6-185.4) | 0.0004 |
| HDL cholesterol (mg/dL) | 49.33 (46.88-51.78) | 49.42 (46.95-52.29) | 48.25 (42.02-54.48) | 0.7 |
| LDL cholesterol (mg/dL) | 110.73 (104.68-116.77) | 116.97 (110.22-123.72) | 87.53 (76.91-98.16) | 0.0001 |
| Triglycerides (mg/dL) | 199.79 (170.08-229.50) | 189.0 (157.5-220.5) | 241.7 (161.4-322.0) | 0.22 |
| AST (UI/mL) | 40.89 (36.01-45.78) | 30.72 (28.30-33.13) | 78.00 (62.01-93.99) | < 0.0001 |
| ALT (UI/mL) | 47.82 (40.54-55.09) | 36.91 (32.03-41.79) | 87.59 (62.16-113.02) | 0.0003 |
| ASTT/ALT ratio | 1.011 (0.949-1.074) | 0.993 (0.927-1.059) | 1.079 (0.913-1.246) | 0.3 |
| GGT (UI/mL) | 83.95 (66.61-101.29) | 59.73 (48.40-71.07) | 174.21 (110.61-237.81) | 0.001 |
| Alkaline phosphatase (UI/mL) | 91.87 (85.09 (98.65) | 90.72 (83.22-98.23) | 98.31 (81.46-115.17) | 0.4 |
| MMP-2 (ng/ml) | 0.538 (0.442-0.654) | 0.482 (0.387-0.600) | 0.867 (0.579-1.300) | 0.02 |
| MMP-3 (ng/mL) | 15.00 (13.24-17.01) | 14.36 (12.47-16.54) | 17.77 (14.45-23.49) | 0.18 |
| MMP-8 (ng/mL) | 0.031 (0.023-0.041) | 0.029 (0.021-0.039) | 0.040 (0.020-0.078) | 0.4 |
| MMP-9 (ng/mL) | 22.49 (18.81-26.90) | 23.19 (19.00-28.31) | 19.94 (13.01-30.58) | 0.5 |
| MMP-10 (ng/mL) | 2.50 (1.66-3.75) | 2.163 (1.487-3.145) | 5.077 (0.938-27.469) | 0.12 |
| TIMP-1 (ng/mL) | 50.56 (45.58-56.08) | 50.84 (45.43-56.88) | 49.49 (37.71-64.94) | 0.8 |
| TIMP-2 (ng/mL) | 8.23 (7.16-9.46) | 7.62 (6.59-8.80) | 11.15 (7.55-16.45) | 0.03 |
| TIMP-4 (ng/mL) | 0.040 (0.030-0.054) | 0.037 (0.027-0.051) | 0.054 (0.026-0.110) | 0. 3 |

1Less than 50 g/d;2Only if detectable;3YFPI: Yearly fibrosis progression index (only in HCV-infected patients), calculated as transient elastometry value divided into the years of estimated HCV infection.Values are expressed as mean (95%CI) or *n* (%) as appropriate. IDU denotes intravenous drug use.

**Table 2 Genotypic frequencies of different single nucleotide polymorphisms according to liver fibrosis ≥ F2 and hepatitis C virus status**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SNP** | **Genotype** | **No fibrosis**  ***n* (%)** | **Fibrosis**  ***n* (%)** | ***P* value** |  | **HIV monoinfected**  ***n* (%)** | **HIV/HCV coinfected**  ***n* (%)** | ***P* value** |
| *MMP-1 -1607 1G/2G* | *1G1G* | 14 (20.3) | 4 (16.7) | 0.9 |  | 9 (16.1) | 9 (24.3) | 0.6 |
|  | *1G2G* | 13 (18.8) | 5 (20.8) |  |  | 12 (21.4) | 6 (16.2) |  |
|  | *2G2G* | 42 (60.9) | 15 (62.5) |  |  | 35 (62.5) | 22 (59.5) |  |
|  |  |  |  |  |  |  |  |  |
| *MMP-8 -799C/T* | *CC* | 30 (27.0) | 6 (22.2) | 0.6 |  | 25 (26.3) | 11 (25.5) | 1 |
|  | *CT* | 47 (42.4) | 10 (37.0) |  |  | 39 (41.1) | 18 (41.9) |  |
|  | *TT* | 34 (30.6) | 11 (40.8) |  |  | 31 (32.6) | 14 (32.6) |  |
|  |  |  |  |  |  |  |  |  |
| *MMP-9 -1562 C/T* | *CC* | 78 (81.2) | 28 (84.8) | 0.6 |  | 61 (83.6) | 45 (80.4) | 0.6 |
|  | *CT* | 18 (18.8) | 5 (15.2) |  |  | 12 (16.4) | 11 (19.6% |  |
|  | *TT* | 0 (0.0) | 0 (0.0) |  |  | 0 (0.0) | 0 (0.0% |  |
|  |  |  |  |  |  |  |  |  |
| *MMP-13 -77A/G* | *AA* | 89 (74.8) | 21 (61.8) | 0.14 |  | 70 (72.9) | 40 (70.2) | 0.7 |
|  | *AG* | 30 (25.2) | 13 (38.2) |  |  | 26 (27.1) | 17 (29.8) |  |
|  | *GG* | 0 (0.0) | 0 (0.0) |  |  | 0 (0.0) | 0 (0.0) |  |
|  |  |  |  |  |  |  |  |  |
| *TNF-α -308 G/A* | *AA* | 11 (11.3) | 4 (11.8) | 0.5 |  | 7 (9.5) | 8 (14.0) | 0.7 |
|  | *AG* | 62 (63.9) | 18 (52.9) |  |  | 46 (62.1) | 34 (59.6) |  |
|  | *GG* | 24 (24.7) | 12 (35.3) |  |  | 21 (28.4) | 15 (26.3) |  |
|  |  |  |  |  |  |  |  |  |
| *CCR5-32* | *wt/wt* | 96 (80.0) | 30 (88.2) | 0.27 |  | 77 (79.4) | 49 (86.0) | 0.3 |
|  | *wt/32* | 24 (20.0) | 4 (11.8) |  |  | 20 (20.6) | 8 (14.0) |  |
|  | *32/32* | 0 (0.0) | 0 (0.0) |  |  | 0 (0.0) | 0 (0.0) |  |

HCV: Hepatitis C virus; HIV: Human Immunodeficiency virus.

**Table 3 Liver fibrosis indexes according to the single nucleotide polymorphisms genotypes**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **SNP** | **Genotype** | **Transient elastometry** | **YFPI** 1 | **APRI** | **Forns** | **FIB-4** |
| *MMP-1 -1607 1G/2G* | *1G1G* | 5.583 (4.600-6.567) | 0.346 (0.215-0.477) | 0.693 (0.192-1.195) | 3.719 (2.860-4.579) | 1.317 (0.723-1.911) |
|  | *1G2G* | 5.911 (4.649-7.174) | 0.470 (0.250-0.691) | 0.488 (0.307-0.670) | 4.253 (3.430-5.077) | 1.092 (0.859-1.324) |
|  | *2G2G* | 8.781 (5.585-11.977) | 0.689 (0.378-1.001) | 0.675 (0.453-0.897) | 4.390 (3.914-4.867) | 1.479 (1.097-1.862) |
|  | *P* value | 0.3 | 0.3 | 0.7 | 0.4 | 0.5 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| *MMP-8 -799C/T* | *CC* | 6.644 (3.736-9.553) | 0.633 (0.074-1.193) | 0.615 (0.411-0.819) | 4.510 (3.920-5.099) | 1.461 (1.058-1.864) |
|  | *CT* | 5.628 (5.060-6.196) | 0.357 (0.275-0.439) | 0.453 (0.347-0.559) | 4.126 (3.701-4.551) | 1.145 (1.014-1.277) |
|  | *TT* | 7.729 (4.540-10.918) | 0.671 (0.376-0.965) | 0.768 (0.447-1.088) | 4.407 (3.825-4.989) | 1.603 (1.081-2.125) |
|  | *P* value | 0.4 | 0.19 | 0.1 | 0.5 | 0.15 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| *MMP-9 -1562 C/T* | *CC* | 7.540 (5.810-9.269) | 0.527 (0.361-0.694) | 0.612 (0.475-0.749) | 4.370 (4.024-4.717) | 1.404 (1.163-1.645) |
|  | *CT* | 11.178 (4.093-18.264) | 0.833 (0.229-1.437) | 0.867 (0.412-1.323) | 5.083 (4.024-6.142) | 2.043 (1.044-3.041) |
|  | *TT* | - | - | - | - | - |
|  | *P* value | 0.14 | 0.16 | 0.16 | 0.11 | 0.065 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| *MMP-13 -77A/G* | *AA* | 7.217 (5.654-8.780) | 0.535-0.358-0.712) | 0.623 (0.480-0.766) | 4.352 (3.993-4.711) | 1.462 (1.185-1.738) |
|  | *AG* | 8.721 (4.646-12.798) | 0.700 (0.291-1.109) | 0.698 (0.426-0.970) | 4.588 (3.998-5.177) | 1.553(1.109-1.998) |
|  | *GG* | - | - | - | - | - |
|  | *P* value | 0.4 | 0.4 | 0.6 | 0.5 | 0.7 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| *TNF-α -308 G/A* | *AA* | 10.073 (1.300-18.849) | 0.656 (0.132-1.180) | 0.693 (0.135-1.250) | 4.331 (3.280-5.383) | 1.584 (0.693-2.475) |
|  | *AG* | 8.086 (5.779-10.394) | 0.552 (0.336-0.767) | 0.675 (0.495-0.855) | 4.603 (4.174-5.033) | 1.575 (1.210-1.940) |
|  | *GG* | 7.514 (4.623-10.404) | 0.620 (0.228-1.012) | 0.701 (0.405-0.997) | 4.221 (3.513-4.929) | 1.466 (1.021-1.910) |
|  | *P* value | 0.7 | 0.9 | 0.99 | 0.6 | 0.9 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| *CCR5-32* | *wt/wt* | 7.852 (5.998-9.707) | 0.590 (0.399-0.783) | 0.664 (0.525-0.803) | 4.439 (4.102-4.775) | 1.513 (1.264-1.762) |
|  | *Wt/32* | 6.521 (4.109-8.934) | 0.545 (0.232-0.858) | 0.539 (0.230-0.847) | 4.391 (3.684-5.098) | 1.335 (0.699-1.971) |
|  | *32/32* | - | - | - | - | - |
|  | *P* value | 0.5 | 0.9 | 0.4 | 0.9 | 0.6 |

1YFPI: Yearly fibrosis progression index (only for HCV-infected patients).Values are expressed as mean (95%CI) in ng/mL.

**Table 4 Metalloproteases and their tissue inhibitors according to the single nucleotide polymorphisms genotypes**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SNP** | **Genotype** | **MMP-2** | **MMP-3** | **MMP-8** | **MMP-9** | **MMP-10** | **TIMP-1** | **TIMP-2** | **TIMP-4** |
|  |  |  |  |  |  |  |  |  |  |
| *MMP-1 1607 1G/2G* | *1G1G* | 0.379 (0.195-0.736) | 16.69 (11.00-25.33) | 0.035 (0.013-0.095) | 19.93 (11.59-34.28) | 1.782 (0.350-9.071) | 41.73 (30.87-56.42) | 6.79 (4.52-10.21) | 0.031 (0.010-0.091) |
|  | *1G2G* | 0.803 (0.409-1.575) | 19.87 (15.13-26.10) | 0.041 (0.012-0.134) | 22.35 (13.37-37.36) | 1.451 (0.417-5.047) | 54.07 (39.44-74.14) | 7.30 (4.49-11.87) | 0.047 (0.020-0.113) |
|  | *2G2G* | 0.609 (0.449-0.826) | 14.72 (11.57-18.73) | 0.027 (0.017-0.042) | 23.65 (17.07-32.76) | 3.345 (1.512-7.399) | 50.34 (42.16-60.10) | 8.39 (6.54-10.76) | 0.032 (0.019-0.053) |
|  | ***P* value** | **0.18** | **0.4** | **0.7** | **0.9** | **0.5** | **0.4** | **0.6** | **0.7** |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| *MMP-8 799C/T* | *CC* | 0.655 (0.473-0.908) | 15.96 (12.94-19.68) | 0.048 (0.025-0.090) | 29.93 (19.94-44.94) | 4.650 (1.354-15.97) | 61.60 (49.94-75.99) | 11.67 (8.59-15.85) | 0.041 (0.023-0.073) |
|  | *CT* | 0.568 (0.409-0.787) | 13.05 (10.22-16.65 | 0.031 (0.018-0.054) | 21.30 (15.50-29.28) | 2.179 (1.201-3.953) | 47.17 (38.80-57.34) | 6.71 (5.50-8.18) | 0.035 (0.021-0.058) |
|  | *TT* | 0.432 (0.280-0.668) | 16.66 (13.35-20.81) | 0.020 (0.015-0.027) | 18.86 (14.03-25.37) | 1.623 (0.938-2.808) | 47.75 (40.75-55.94) | 8.12 (5.89-11.20) | 0.050 (0.030-0.084) |
|  | ***P*  value** | **0.3** | **0.3** | **0.09** | **0.17** | **0.17** | **0.11** | **0.01** | **0.6** |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| *MMP-9 1562 C/T* | *CC* | 0.495 (0.385-0.637) | 15.42 (13.11-18.14) | 0.034 (0.024-0.049) | 22.39 (17.79-28.19) | 2.405 (1.462-3.957) | 52.23 (46.17-59.07) | 8.39 (7.18-9.80) | 0.037 (0.026-0.053) |
|  | *CT* | 1.031 (0.853-1.246) | 18.15 (13.99-23.54) | 0.020 (0.008-0.047) | 22.97 (14.77-35.71) | 5.960 (0.666-53.34) | 46.55 (33.82-64.07) | 8.18 (4.40-15.21) | 0.058 (0.025-0.137) |
|  | *TT* | - | - | - | - | - | - | - | - |
|  | *P* value | 0.02 | 0.3 | 0.2 | 0.9 | 0.2 | 0.4 | 0.9 | 0.3 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| *MMP-13 77A/G* | *AA* | 0.522 (0.419-0.650) | 15.62 (13.47-18.13) | 0.030 (0.022-0.041) | 23.06 (18.64-28.54) | 2.337 (1.380-3.957) | 53.30 (47.05-60.38) | 8.54 (7.21-10.12) | 0.050 (0.036-0.069) |
|  | *AG* | 0.683 (0.458-1.017) | 14.37 (11.10-18.59) | 0.037 (0.019-0.071) | 22.96 (16.01-32.93) | 2.685 (1.215-5.931) | 48.10 (39.30-58.88) | 8.07 (6.09-10.68) | 0.031 (0.016-0.058) |
|  | *GG* | - | - | - | - | - | - | - | - |
|  | *P*  value | 0.22 | 0.6 | 0.6 | 0.98 | 0.8 | 0.4 | 0.7 | 0.14 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| *TNF-α 308 G/A* | *AA* | 0.730 (0.325-1.639) | 21.40 (14.72-31.13) | 0.039 (0.010-0.156) | 17.42 (8.16-37.21) | 0.804 (0.369-1.755) | 57.25 (37.28-87.92) | 13.14 (8.72-19.80) | 0.111 (0.054-0.227) |
|  | *AG* | 0.558 (0.429-0.727) | 14.65 (11.96-17.95) | 0.031 (0.020-0.050) | 23.41 (17.78-30.83) | 4.460 (2.097-9.486) | 51.62 (43.92-60.66) | 8.39 (6.71-10.48) | 0.043 (0.028-0.064) |
|  | *GG* | 0.453 (0.281-0.732) | 16.54 (13.36-20.47) | 0.027 (0.018-0.040) | 23.62 (17.25-32.34) | 1.998 (0.879-4.542) | 46.63 (39.84-54.59) | 7.04 (5.36-9.26) | 0.023 (0.012-0.045) |
|  | *P* value | 0.4 | 0.24 | 0.8 | 0.7 | 0.065 | 0.6 | 0.1 | 0.02 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| *CCR5-32* | *wt/wt* | 0.601 (0.484-0.747) | 15.75(13.71-18.09) | 0.031 (0.022-0.043) | 21.55 (17.50-26.53) | 2.441 (1.482-4.020) | 50.72 (44.83-57.38) | 8.10 (6.95-9.45) | 0.041 (0.029-0.057) |
|  | *wt/32* | 0.431 (0.286-0.652) | 13.64 (9.87-18.85)) | 0.032 (0.020-0.051) | 27.15 (18.55-39.73) | 2.662 (1.222-5.801) | 52.01 (42.66-63.42) | 9.44 (6.49-13.72) | 0.043 (0.021-0.088) |
|  | *32/32* | - | - | - | - | - | - | - | - |
|  | *P*  value | 0.15 | 0.4 | 0.9 | 0.3 | 0.9 | 0.9 | 0.4 | 0.9 |

Values are expressed as mean (95%CI) in ng/mL.

**Table 5 Correlations among metalloproteases, their tissue inhibitors and liver fibrosis parameters**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **MMP-3** | **MMP-8** | **MMP-9** | **MMP-10** | **TIMP-1** | **TIMP-2** | **TIMP-4** | **TE 1** | **YFPI 2** | **APRI** | **Forns** | **FIB-4** |
| MMP-2 | 0.19 (0.04) | 0.21 (0.02) | 0.21 (0.02) | 0.20 (0.09) | 0.29 (0.002) | 0.29 (0.002) | 0.35 (0.0002) | 0.23 (0.016) | 0.30 (0.058) | 0.20 (0.03) | 0.19 (0.04) | 0.20 (0.03) |
|
| MMP-3 |  | 0.29 (0.0006) | 0.30 (0.0006) | 0.15 (0.19) | 0.33 (0.0001) | 0.27 (0.002) | 0.38 (< 0.0001) | 0.09 (0.3) | 0.001 (0.99) | 0.16 (0.06) | 0.16 (0.06) | 0.10 (0.24) |
|
| MMP-8 |  |  | 0.69 (< 0.0001) | 0.25 (0.02) | 0.71 (< 0.0001) | 0.40 (< 0.0001) | 0.24 (0.005) | -0.02 (0.8) | -0.15 (0.3) | -0.01 (0.9) | 0.03 (0.8) | -0.03 (0.7) |
|
| MMP-9 |  |  |  | 0.21 (0.06) | 0.71 (< 0.0001) | 0.16 (0.06) | 0.21 (0.01) | -0.21 (0.01) | -0.32 (0.02) | -0.17 (0.047) | -0.11 (0.2) | -0.20 (0.02) |
|
| MMP-10 |  |  |  |  | 0.12 (0.3) | 0.22 (0.051) | 0.11 (0.3) | 0.01 (0.9) | -0.09 (0.7) | 0.12 (0.3) | -0.08 (0.5) | -0.03 (0.8) |
|
| TIMP-1 |  |  |  |  |  | 0.40 (<0.0001) | 0.39 (<0.0001) | 0.02 (0.8) | -0.04 (0.8) | -0.02 (0.8) | 0.07 (0.4) | 0.01 (0.9) |
|
| TIMP-2 |  |  |  |  |  |  | 0.33 (0.0001) | 0.28 (0.0009) | 0.25 (0.08) | 0.35 (< 0.0001) | 0.20 (0.018) | 0.36 (< 0.0001) |
|
| TIMP-4 |  |  |  |  |  |  |  | 0.11 (0.2) | 0.03 (0.8) | 0.14 (0.12) | 0.06 (0.5) | 0.19 (0.03) |
|
| TE |  |  |  |  |  |  |  |  | 0.94 (< 0.0001) | 0.75 (< 0.0001) | 0.59 (< 0.0001) | 0.82 (< 0.0001) |
|
| YFPI1 |  |  |  |  |  |  |  |  |  | 0.63 (< 0.0001) | 0.51 (0.0001) | 0.74 (< 0.0001) |
|
| APRI |  |  |  |  |  |  |  |  |  |  | 0.55 (< 0.0001) | 0.90 (< 0.0001) |
|
| Forns |  |  |  |  |  |  |  |  |  |  |  | 0.67 (< 0.0001) |
|

1TE denotes transient elastometry; 2YFPI denotes yearly fibrosis progression index (only for HCV-infected patients). Values are expressed as *r* (*P* value).

**Table 6 Independent predictors of different fibrosis indexes**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **TE** | **APRI** | **Forns** | **FIB-4** | **YFPI** |
| Higher MMP-2 levels | 0.001 | 0.0001 | 0.03 | 0.0009 | 0.004 |
| Higher TIMP-2 levels | 0.016 | 0.0001 | 0.024 | 0.0002 | - |
| Lower MMP-9 levels | 0.023 | 0.016 | - | 0.03 | 0.043 |
| Lower current CD4 counts | - | 0.032 | 0.037 | 0.043 | - |
| Older age | - | - | 0.004 | 0.05 | - |
|  |  |  |  |  |  |
| % of the index accounted for by the model | 20.0% | 35.3% | 23.3% | 33.5% | 20.7% |

TE denotes transient elastometry and YFPI yearly fibrosis progression index (only for HCV-infected patients). Numbers represent *P* values.