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RELATIONSHIP BETWEEN SERUM CONCENTRATIONS OF TYPE III PROCOLLAGEN, HYALURONIC ACID AND HISTOPATHOLOGICAL FINDINGS IN THE LIVER OF HCV-POSITIVE BLOOD DONORS

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ABSTRACT – ***Background*** - Serologic markers have been proposed for monitoring hepatic fibrosis in chronic liver disease. Among fibrosis markers, type III procollagen (PIIIP) and hyaluronic acid have been studied in these patients. ***Aim*** - To evaluate the association between these serum markers with histological findings. ***Methods*** - A prospective cross-sectional study was carried out with HCV-positive blood donors. The studied population included men and women whose age ranged from 18 to 60 years, with elevated liver function tests [ALT levels ≥ 1.5 times the normal value and alterations of two or more of the following: any changes in the levels of ALT, aspartate aminotransferase, conjugated bilirrubin, gammaglobulin, gammaglutamyltranspeptidase, albumin, platelet count; alkaline phosphatase levels ≥1.5 times the normal value, or prothrombin time below 70% and above 60%]. Fourty-nine patients were submitted to liver biopsy, blood analysis of PIIIP, hyaluronic acid, besides liver function tests. ***Results*** - Liver function tests were not associated with tissular fibrosis, as assessed by ALT (≥1.5 times above normal, fibrosis risk=18.8%; <1.5 times, 11.8%). Elevated PIIIP was correlated with 66.7% chance of fibrosis, whereas normal levels, 9.3%. Hyaluronic acid, when elevated, gave a chance of 33.3% of fibrosis; when normal, 12.5%. ***Conclusions*** - There was no association between liver function tests, hyaluronic acid and fibrosis. However, PIIIP was related with liver fibrosis. Maybe, this marker should be useful to assess fibrosis in patients with chronic hepatitis C.

HEADINGS – Hepatitis C, chronic. Liver cirrhosis. Collagen type III. Hyaluronic acid. Blood donor.

INTRODUCTION

Prognosis of chronic hepatitis C is close related to the development of fibrosis, which is the most important factor for estimating clinical outcome and determining therapeutic strategy, especially interferon therapy in this setting[23]. Liver biopsy is the current way for diagnosing liver fibrosis, necrosis and inflammation. However, it has potential complications, and therefore, it is not performed repeatedly. Thus, there is a need for non-invasive methods in attempt of assessing the severity of hepatic fibrosis, and. several noninvasive markers have been studied[5, 15, 21, 23]. In fact, serum assays for products of matrix synthesis or degradation and the enzymes involved in these process have been investigated as surrogate markers of liver fibrosis in a number of studies[13,21,29]. Non-invasive biochemical markers are being employed to quantify tissue damage and to monitor the progression of fibrosis[15, 25], and the combination of hepatic fibrogenesis serum markers has been shown to be a useful non-invasive method for evaluating hepatic histology. Among the single fibrosis markers, hyaluronic acid (HA) and type III procollagen (PIIIP) levels have been studied in patients with chronic liver diseases[13, 21, 23, 25].

PIIIP is a serum marker of active fibrogenesis, which plays a crucial role in development of cirrhosis, and liver stellate cells, activated to myofibroblasts, expressing alpha-smooth muscle actin are responsible for deposition of fibrous matrix[7]. PIIIP seems to be related mainly with histological activity in chronic hepatitis and their levels seem to be especially high in the presence of active hepatic diseases, such as chronic hepatitis and active cirrhosis[15, 21, 23, 29, 31]. PIIIP may also be used to evaluate therapeutic efficacy of antifibrotic drugs as D-penicillamine and interferon in cases of primary biliary cirrhosis[28] or chronic viral hepatitis[7, 30, 31].

HA is a glycosaminoglycan synthesized by mesenchymal cells and degraded by hepatic sinusoidal cells through a specific receptor-mediated process[23]. Their serum levels

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increase directly with the progression of a given chronic hepatic disease. Although HA has been correlated with fibrosis, this is not truly associated with inflammation and necrosis[3, 12, 16, 18, 23, 30, 31].

Still, there is a dearth of studies evaluating the association of PIIIP and HA with fibrosis in hepatitis C virus (HCV) patients. Therefore, our aim was to assess the relation of serum PIIIP and HA levels, and LFT, with liver fibrosis in HCV-positive blood donors.

METHODS

A prospective study was carried out at "Hospital de Clínicas de Porto Alegre", Porto Alegre, RS, Brazil. Two hundred and forty HCV-positive blood donors were examined, and 62 fulfilled the criteria of inclusion. Among these, 13 were subsequently lost, as result of a scant liver biopsy sample or blood storage failures.

Were included individuals with alanine aminotransferase (ALT) levels ≥1.5 times the normal value and/or alterations of two or more of the following: any changes in ALT, aspartate aminotransferase (AST), conjugated bilirrubin (CB), gammaglobulin, gammaglutamyltranspeptidase (GGT), albumin, platelet count, alkaline phosphatase (AP) ≥1.5 times the normal value, and prothrombin time (PT) below 70 % and above 60%. Exclusion criteria were: HBV or HIV co-infection, PT below 60% (not responsive to reposition of vitamin K), platelet count below 80.000/mm³, presence of hyperdense or cystic hepatic lesions indicated by ultrasonography, and biopsy fragments smaller than 1 cm and with fewer than three portal tracts.

Merck commercial kits were used to determine in duplicate serum concentrations of ALT, AST, AP, albumin, GGT, total bilirubin (TtB), and conjugated bilirrubin, using an automatic analyzer. Serum gammaglobulin was separated by electrophoretic migration and quantified by optic densitometry. Serum PIIIP concentration was determined through radioimmunoassay (Kit Orion Diagnostica Procollagen PIIIP, Finland [I^{125}]. The normal reference interval for adults ranged from 1.7 to 4.2 µg/L, for both female and male patients.

Serum concentration of HA was determined by radiometric assay (Pharmacia HA test). The normal reference interval for adults ranged from 7 to 65 µg/L. HCV ELISA 2 immunoenzymatic test (Abbott HCV EIA II) was used.

All patients were submitted to blinded percutaneous liver biopsy with a Trucut needle number 16 to assess fibrosis. Patients were also submitted to collection of a 10 mL blood sample. Serum was stored in a duplicate at -20° C. Fragments were fixed with 10% formalin, and later stained with hematoxylin-eosin, Gomori trychrome, picrosirius red, and reticulin. METAVIR was the criteria used for grading and staging biopsies[1]. A1 and F1 were the minimal criteria considered either for the activity and stage, respectively.

Statistical analysis

In this study, liver biopsy was the gold standard for demonstrating fibrosis, and were compared to PIIIP and HA. Sensitivity, specificity, positive predictive value, negative predictive value, and [1- negative predictive value] was calculated. The Fleiss method was used to calculate a 95% confidence interval (CI) for liver biopsy. Moreover, the association of each potential diagnostic variable with fibrosis and with chronic hepatitis was calculated using the chi-square test (χ^2), with Yates' correction or Fisher's exact test when necessary. Effect was estimated using relative risk (RR), and 95% confidence intervals. The significance level for this study was $\alpha = 0.05$. Data were processed and analyzed using Epi- Info 6.0 and Statistical Package for the Social Sciences (SPSS) for Windows.

This study was approved by HCPA Ethics Committee. All patients gave their signed consent.

RESULTS

Forty-nine patients were studied, ages ranging from 18 to 60 years [average ± standard deviation (SD) = 34.7 ± 9.5 years]. 37 patients (75.5%) were male, and 12 (24.5%) female. Regarding race, 43 patients (87.8%) were European-derived whites, and 6 patients (12.2%) were non-white.

The average number of portal tracts obtained was 7.4 ± 4.4 (average ± SD) and the findings were normal in 6 patients (12.2%); macrovesicular steatosis, in other 9 (18.4%), minimal hepatitis, 9 (18.4%), and 25 specimens with chronic hepatitis. Regarding the last group, Table 1 shows their findings.

Table 2 shows the results of biochemical, hematological and serum markers of fibrosis. Most of the patients had elevated AST or ALT. When analyzing PIIIP and HA, 12.2% and 18.4% , respectively, had elevated levels.

TABLE 1. Distribution of cases with relation to grading and staging of fibrosis*

	Staging					
Grading	F0	F1	F2	F3	F4	Total
A1	8	1	0	0	0	9 (36%)
A2	9	3	2	0	1	15 (60%)
A3	0	0	1	0	0	1 (4%)
A4	0	0	0	0	0	0
Total	17 (68%)	4 (16%)	3 (12%)	0	1 (4%)	25 (100%)

*As diagnosed by biopsy

TABLE 2. Results of biochemical, hematological and serum markers of fibrosis

Variable	Reference value	%*	Average ± SD
Alanine aminotransferase	0-22 µ/L	85.7	52.5 ± **37.9 µ/L**
Aspartate aminotransferase	4-18 µ/L	73.5	29.2 ± 17.7 µ/L
Total bilirubin	0-1.2 mg/L	10.2	0.90 ± 0.39 mg/L
Conjugated bilirubin	0-0.2 mg/Ll	16.3	0.15 ± 0.11 mg/L
Alkaline phosphatase	0-170 µ/L	10.2	112.4 ± **47.8 µ/L**
Gammaglutamyl-transpeptidase			
Men	6-28 µ/L	50	47.9 ± 59.2 µ/L
Woman	4-18 µ/l		
Prothrombin time	80%-100%	55.1	77.9% ± 15.7%
Platelets	140.000-340.000 mm³	8.2	205.217 ± 67.352 mm³
Albumin	3.5-5.6 g%	4,1	4.25 ± **0.37 g%**
Gammaglobulin	0.7 - 1.4 g%	49	1.44 ± **0.32 g%**
Type III procollagen	1.70 - 4.20 **µmg/L**	12.2	2.85 ± **1.29 µg/L**
Hyaluronic acid	7-65 µg/L	18.4	47.04 ± 22.03 µg/L

*Rate of patients with altered results

The average ± SD of all biochemical variables was evaluated using the Student test, taking into consideration the presence, or absence, of fibrosis as defined by biopsy (Table 3). As can be seen in Table 4, only PIIIP was related to the presence of fibrosis ($P = 0.041$). HA revealed a tendency towards association with fibrosis (average 60.7 ± 24.6 µg/L), but without statistical significance ($P = 0.054$). Similarly, AST revealed a tendency towards association with fibrosis (average 39.9 ± 22.8 µg/L), again without statistical significance ($P = 0.062$). The remaining variables (ALT, TtB, DB, AP, GGT, PT, platelets, albumin, and gammaglobulin) were not associated with fibrosis. Table 5 shows specificity, sensibility and prognostic predictive values that were obtained.

DISCUSSION

HCV infection has a worldwide distribution, with a prevalence of about 3%[27]. The frequency of anti-HCV in blood donors

TABLE 3. Results of the variables (average * SD) studied in relation to presence or absence of fibrosis

Variables	Fibrosis (n=8) (average ± SD)	Fibrosis (n=41) (average ± SD)	*P*
Alanine aminotransferase	68.6 ± 40.5 µ/L	49.3 ± 37.1 µ/L	0.190
Aspartate aminotransferase	39.9 ± 22.8 µ/L	27.1 ± 16.1 µ/L	0.062
Total bilirubin	0.88 ± 0.28 µ/L	0.90 ± 0.41 µ/L	0.857
Conjugated bilirubin	0.16 ± 0.12 µ/L	0.15 ± 0.11 µ/L	0.816
Alkaline phosphatase	123.0 ± 31.6 µ/L	122.2 ± 50.7 µ/L	0.967
Gammaglutamyl-transpeptidase	45.6 ± 32.0 µ/L	48.4 ± 63.7 µ/L	0.904
Prothrombin time	73.1% ± 14.1%	81.4% ± 15.7%	0.174
Platelets	173.000 ± 54.689/mm3	212.0 ± 68.408/mm^3	0.138
Albumin	4.1 ± 0.4 mg%	4.3 ± 0.4 mg%	0.211
Gammaglobulin	1.62 ± 0.4 mg%	1.41 ± 0.3 mg%	0.112
Type III procollagen	4.42 ± 2.12 µg/L	2.54 * 0.78 µg/L	0.041*
Hyaluronic acid	60.7 ± 24.6 µg/L	44.4 ± 20.8 µg/L	0.054

*P<0.05

TABLE 4. Serum PIIIP and HA levels and their relation with fibrosis

PIIINP(VN:1,70-4,20 µg/L)	**Fibrosis**		**Total**
	F1-F4	**F0**	
≥4.20 **µg/L**	4 (66,7%)	2	6
≤4.19 **µg/L**	4 (9,3%)	39	43
Total	8	41	49*
HA (VN: 7-65 **µg/l)**	**Fibrosis**		**Total**
	F1-F4	**F0**	
≥65.0 µg/L	3 (33.3%)	6	9
≤64,9 **µg/L**	5 (12,5%)	35	4
			0
Total	8	41	49**

PIIIP: type III procollagen; HA: hyaluronic acid; RR: relative risk
*RR = 7.17 (IC 95%: 2.41-21.35); P = 0.004
**RR = 2.67 (IC 95%: 0.78-9.17); P = 0.151

TABLE 5. Accuracy of ALT, PIIIP, and HA association taking into consideration the presence of fibrosis

Variable	Sensitivity (CI 95%)	Specificity (CI 95%)	Positive Predictive value (CI 95%)	Negative Predictive value (CI 95%)	1-negative Predictive value (CI 95%)	RR* (CI 95%)
Alanine aminotransferase ≥^{3} 33 **µ/L**	75.0% (35.6-95.5)	36.6% (22.6-53.1)	18.8% (7.9-37.0)	88.2% (62.3-97.0)	11.8% (2.1-37.8)	1.59 (0.36-7.06)
Type III procollagen ≥^{3}4.20 mg/L	50% (17.4-82.6)	95.1% (82.2-99.2)	66.7% (24.1-94.0)	90.7% (76.9-97.0)	9.3% (3.0-23.1)	7.17 (2.41-21.35)
Hyaluronic acid ≥^{3}65.0 mg/L	37.5% (10.2-74.1)	85.4% (70.1-93.9)	33.3% (9.0-69.1)	87.5% (72.4-95.3)	12.5% (4.7-27.6)	2.67 (0.78-9.17)

* Relative risk

varies from about 1% to 2%, according to the geographic area and the population studied[24]. HCV is the pathogen related with 80% to 90% post-transfusional hepatitis[10, 26].

In the present study, the main laboratorial alterations occurred in ALT and AST levels that were above normal in 85.7% and 73.5% of the blood donors, respectively (Table 2). DOR-MOHAMADI et al.[4] observed elevated ALT levels in 65% of the studied population, and other studies found an even lower prevalence of elevated ALT levels (5% to 40%)[19,24].

Our results indicated that the levels of GGT were above normal in 50% of patients (Table 2), which agrees with other studies[11, 20]. Our results also indicated that 49% of patients had elevated levels of gammaglobulin, and PT was reduced in 55.1% of our patients. All of these responded to vitamin K replacement, thus allowing the performance of liver biopsy. Interestingly, ROSSINI et al.[22] verified that elevated levels of gammaglobulin were predictive of viremia.

Only 6 out of 49 patients (12.2%) presented a normal hepatic tissue at biopsy. Also we observed that 18.4% of patients had steatosis, and that 18.4% had minimal hepatitis. SANTANA et al.[24] also studying blood donors with normal and abnormal ALT, observed normal histological findings in 2.3% of patients, and minimal alterations in 16% of patients. Twenty five patients (51%) had chronic hepatitis, most of them grading from A1 to A2. Seventeen out of 25 patients (68%) had fibrosis, while other studies reported results ranging from 41% to 90%[22, 26]. Interestingly, one of our patients showed cirrhosis.

Hepatic histology is considered the gold standard for evaluating histological lesions, disease activity (necrosis and hepatocyte inflammation), and fibrosis. The serum markers of fibrosis, PIIIP and HA, have been suggested as parameters for monitoring fibrogenesis[6, 7, 25, 31]. Many patients with acute and chronic liver diseases present high serum PIIIP concentration. Most importantly, serum PIIIP concentration reflects inflammatory activity and active fibrogenesis[28]. Consequently, it would be possible to use serum PIIIP levels to predict the response to chronic hepatitis treatment with interferon[2, 6, 9, 17].

Considering the presence of fibrosis and serum PIIIP levels for the 49 patients in this study, 6 patients (12.2%) presented levels above normal, and the remaining 43 patients (87.8%) presented normal levels. Of the six patients with above normal serum levels, four (66.7%) had fibrosis; and of the patients with normal serum levels, four (9.3%) had fibrosis ($P = 0.004$). According to our data, there was a 66.7% chance that patients with above normal serum PIIIP levels had fibrosis, and only a 9.3 % chance that patients with normal serum PIIIP levels had fibrosis. Thus, our results suggested that there is an association between high levels and fibrosis.

Serum HA levels increase in patients with chronic hepatitis C; and this is related to the degree of hepatic fibrosis, and therefore HA could be used as a non-invasive method for the evaluation of the extension of fibrosis in chronic hepatitis C patients[22]. Recent studies have reported that HA is better than PIIIP as a marker of fibrosis because it allows a better distinction of patients with extensive fibrosis in relation to patients with mild or no fibrosis. Such studies also report that HA is not correlated to histopathological indexes of hepatic inflammation or necrosis[5, 12, 23]. Thus, HA may be very useful for monitoring anti-fibrosis therapeutics in patients with chronic hepatitis being treated with interferon[6, 8, 14].

Concerning the correlation between serum HA levels and fibrosis, in the present work we observed that 9 patients (18.4%) had above normal serum levels, and that the remaining 40 patients (81.6%) had normal HA levels. Three of the 9 patients (33.3%) with above normal serum levels had fibrosis, whereas 5 of the 40 (12.5%) patients with normal serum levels had fibrosis ($P = 0.151$). Thus, there was no association of HA with the presence of fibrosis. Patients with above normal serum HA levels had a 33.3% chance of having fibrosis, and patients with normal serum levels still had a 12.5% chance of having fibrosis. Consequently, the present results do not support the hypothesis that HA is good test for predicting fibrosis.

In conclusion, chronic hepatitis was present in 51% of the blood donors studied, and there was a predominance of minimal to mild histological activity. Isolated fibrosis was present in 16.3% of patients. PIIIP was associated to the presence of fibrosis, but HA was not. Liver biopsy still is the main tool for identifying fibrosis in patients with chronic hepatitis C, but PIIIP, a non-invasive serum marker, should also be useful in this population.

Camacho VRR, Silveira TR, Oliveira JR, Barros SGS, Cerski CTS. Relação entre concentrações séricas de procolágeno tipo III, ácido hialurônico com achados histopatológicos do fígado em doadores de sangue anti-HCV positivos. Arq Gastroenterol. 2007;44(2):118-22.

RESUMO – ***Racional -*** Marcadores sorológicos têm sido propostos para monitorar fibrose hepática em doença crônica do fígado. Dentre os marcadores de fibrose, ácido hialurônico e procolágeno tipo III têm sido estudados nestes pacientes. ***Objetivo -*** Avaliar a associação de marcadores séricos de fibrose com achados histológicos. ***Métodos -*** Foi realizado estudo transversal prospectivo em doadores de sangue anti-HCV positivos. A população estudada incluiu homens e mulheres com idade entre 18-60 anos com provas de função hepática alteradas (níveis de alanina aminotransferase ≥1.5 vezes do normal e alterações de dois ou mais dos seguintes: qualquer alteração nos níveis de alanina aminotransferase, aspartato aminotransferase, bilirrubina conjugada, gamaglobulina, gamaglutamiltranspeptidase, albumina, plaquetas, níveis de fosfatase alcalina ≥1,5 vezes o valor normal, tempo de protrombina abaixo de 70% e acima de 60%). Quarenta e nove pacientes foram submetidos a biopsia hepática e coleta de sangue para análise de procolágeno tipo III, ácido hialurônico e provas funcionais hepáticas. ***Resultados -*** Não houve relação entre elevação de provas de função hepática e a presença de fibrose - ALT (≥1,5 vezes acima do normal, risco de fibrose = 18,8%; <1,5 vezes, 11,8%). Procolágeno tipo III elevado foi correlacionado com 66,7% chances de fibrose, enquanto nível normal, 9,3%. Ácido hialurônico, quando elevado, demonstrou chance de 33,3% de fibrose; quando normal, 12.5%. ***Conclusões -*** Não houve associação entre provas de função hepática, ácido hialurônico e fibrose, mas houve entre esta última e procolágeno tipo III. Talvez este marcador possa ser útil para avaliar fibrose em pacientes com hepatite crônica pelo vírus C.

DESCRITORES – Hepatite C crônica. Cirrose hepática. Colágeno tipo III. Ácido hialurônico. Doadores de sangue.

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