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ORIGINAL ARTICLE

CORRELATION AMONG THREE NON-INVASIVE METHODS (APRI, FIB-4 AND TRANSIENT ELASTOGRAPHY) TO EVALUATE LIVER FUNCTION AND STIFFNESS IN PATIENTS WITH VIRAL HEPATITIS C OR SCHISTOSOMIASIS MANSONI

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ABSTRACT

Both chronic viral hepatitis and schistosomiasis are potentially serious causes of liver fibrosis. Several studies suggest, however, that hepatic fibrosis may be reversible, which highlights the importance, not only of early diagnosis, but, above all, observing this while monitoring the disease. This study aimed to evaluate the correlation between three non-invasive methods for classification of hepatic fibrosis in patients with viral hepatitis or schistosomiasis. The sample consisted of 45 patients with chronic hepatitis with HCV and 17 with schistosomiasis. Medical records were analyzed for data collection related to sex, body mass index (BMI) and laboratory testing for biochemical markers. The evaluation was carried out by means of the following hepatic diagnostic methods: APRI, FIB-4 and Transient Elastography (Fibroscan), and the subsequent correlation analysis by Spearman test (r). There was a predominance of males among patients with HCV (64.4%). HCV patients also presented the highest average age (54.8 years) and high levels of AST and ALT. Positive correlation was noted between APRI and FIB-4 results in patients of both groups; positive correlation between APRI and Transient Elastography in patients with HCV; and positive correlation between FIB-4 and Elastography in patients with HCV or schistosomiasis. Our data suggest that the non-invasive methods for diagnosis and monitoring of hepatic fibrosis present a high degree of acceptance, especially in patients with HCV.

KEY WORDS: Liver Diseases; fibrosis; diagnosis; elastography

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INTRODUCTION

Viral hepatitis is a serious public health problem in Brazil and in many countries around the world. It is caused by different etiological agents, resulting in high incidences of morbidity and mortality, especially in endemic areas with insufficient care services for carriers of the infection (Santos et al., 2008; WHO, 2017). According to epidemiological data, 170 million people are estimated carriers of hepatitis C virus (HCV). In addition, 1.75 million new HCV infections were estimated for 2015. WHO also estimates that in 2015, 71 million people had HCV infection, accounting for 1% of world population (Santos et al., 2008; Scaraveli et al., 2011; WHO, 2017).

Schistosomiasis mansoni (schistosomiasis) is a serious parasitic disease caused by *Schistosoma mansoni* which is waterborne and evolves chronically. It occurs in 78 countries, with an estimated 249 million infected people and 700 million others in hazardous areas (WHO, 2014). In Brazil, data indicate a prevalence of 8 million people with the disease (GVE/ MS, 2009; Sarvel et al., 2011). In an endemic region, the intestinal-tract-type is the most frequently found (more than 90% of the cases), while the hepatosplenic-type is the most aggravating form of the disease, being associated with splenomegaly, hepatic fibrosis and portal hypertension, found in 4% to 10% of schistosomal patients (Morais et al., 2010; Silva & Domingues, 2011, WHO, 2014).

It is important to note also that both pathologies (viral hepatitis and schistosomiasis) can evolve with hepatic fibrosis. In this context, the accurate assessment of fibrosis has become increasingly important for therapeutic decision-making, prognosis and disease follow-up. New studies suggest that hepatic fibrosis may be reversible (Andrade, 2005; Chang et al. 2010), increasing the importance of not only the fibrosis diagnosis but also following it over time (Papastergiou et al., 2012). Liver biopsy is still the gold standard for evaluation (Bedossa & Carrat, 2009), however, despite frequent application in clinical practice, it is an invasive procedure that presents risks and complications (Ding et al., 2015; Rockey et al., 2009). Accurate, reproducible and easy to apply techniques are required for monitoring fibrosis (Papastergiou et al., 2012). Among these methods, the serum markers (APRI and FIB-4) are highlighted as well as imaging methods (Fibroscan) that can be used separately or combined into algorithms to increase accuracy (Bedossa & Carrat, 2009; Bonder & Afdhal, 2013; Schiavon et al., 2014). Schiavon et al. (2014) suggest that hepatic fibrosis biological markers allow an objective result interpretation and significantly reduce the bias risk of tester variability that occurs in liver biopsy, along with the advantage of not being invasive.

Transient Elastography (Fibroscan) is an excellent liver cirrhosis diagnostic method, presenting an AUROC curve of 94% and for severe fibrosis an AUROC curve of 89% (Castera et al., 2005), accurately determining which are F3 and F4 patients (Arena et al., 2008). However there are situations where the use of fibroscan is not indicated, namely: cases of IQR (Interquartile range) numbering 30%, operator inexperience, insufficient number of valid measurements, anthropometric factors (BMI, thickness of the rib cage, waist circumference, distance between skin and liver capsule) (Bonder & Afdhal, 2014; Cardoso et al., 2012).

In addition, studies have shown that there is an overlap of values when analyzing lower fibrosis degrees (F0, F1 and F2) (Arena et al., 2008; Castera, 2011), which is a limiting factor. However, the limitations of the various methods can be reversed when applying them simultaneously. Poynard et al. (2008) have shown that fibroscan featured a similar accuracy to fibrotest. Castera et al. (2005) showed that the combination of methods, especially Fibroscan and fibrotest, managed to improve the AUROCs for three degrees of fibrosis assessed (F2, F3 and F4). In this context, the objective of this study was to evaluate the correlation between three non-invasive methods (APRI, FIB-4 and Elastography) for diagnosis of liver fibrosis in patients with viral hepatitis and schistosomiasis.

MATERIAL AND METHODS

Patients, data collection and ethical considerations.

A cross-sectional epidemiological study was carried out, in which 93 patients seen in the Hepatology outpatient clinic of the Hospital Universitário – Universidade Federal de Sergipe (HU-UFS) who had undergone liver digital Elastography in 2015 were studied. The sample consisted of 45 patients with chronic hepatitis by HCV and 17 with schistosomiasis in the chronic phase (12 with the hepatointestinal form and 5 with the hepatosplenic form).

In addition, each chart was analyzed for the collection of data regarding sex, body mass index (BMI) and laboratory tests (aspartate aminotransferase-AST, alanine aminotransferase-ALT and gamma glutamil transferase-γGT) carried out in the laboratory of clinical analyses of the University Hospital (HU-UFS). No exclusion criteria were used among the patients selected.

Patients were invited to participate spontaneously. They were informed about the nature of the study, the methods and why the research was being performed. Those who agreed to participate in the study signed an informed consent form. The research was approved by the Research Ethics Committee of the University Hospital (HU) of the Federal University of Sergipe/UFS, under approval number 1711796 and CAAE number 58538116900005546.

Assessment of hepatic fibrosis by APRI and FIB-4 methods

The traditional laboratory tests to measure the degree of hepatic fibrosis APRI and FIB-4 were applied according to the formulas taken from the original publications as follows.

The values for APRI were calculated through the formula: [(AST /AST normalcy top limit) / (number of platelets (10^9/L)] × 100. In this calculation, the numerator corresponds to the ratio of the value of AST (U/L) and the reference value of AST (using -38 as the reference value of HU/UFS). The denominator refers to the quantity of platelets (109/L) for each patient. The end result of the equation is multiplied by 100. The value found is classified, according to the Metavir parameters: F0 ≤ 0.22; F10.22-0.39; F20.39-0.88; F30.88-2.48; F4 2.48 (Ferenci et al., 2014). The APRI is one of the simplest scores used for diagnosing liver fibrosis and cirrhosis with acceptable accuracy. The value of the upper limit of normal for all calculations was 38.

For the calculation of the FIB-4 a combination of four variables was used: AST, ALT, age and number of platelets. These variables were applied to the formula: FIB-4 = [age (years) × AST (IU/L)] / [number of platelets (10^9/L) × √ALT (IU/L)], in which the numerator corresponds to the multiplication of the patient's age (in years) by the AST (U/L) value and the denominator is the multiplication of the platelets value (10^9/L) by the square root of ALT (U/L). The value found is classified, according to the Metavir parameters: F0 ≤ 0.38; F1 > 0.38 - 0.68; F2 > 0.68 - 1.4; F3 > 1.4 - 3.2; F4 > 3.2 (Ferenci et al., 2014).

Liver stiffness measurement by Transient Elastography (Fibroscan)

The liver stiffness of each patient was measured by Transient Elastography in 2015. This Ultrasound method evaluated liver right lobe rigidity through the intercostal spaces at a subcutaneous depth of 25-45 mm, with the patient lying supine with their right arm in maximum abduction. The transducer was covered with gel to reduce resistance and the elasticity value was obtained from the average of 10 liver analyses. The results were expressed in kilopascals (Kpa).

Statistical analysis

The analysis of the demographic and clinical data of the patients was performed by the Fisher exact test. Levels of biochemical markers were analyzed and the different groups compared. Data are shown as mean and standard deviation and minimum and maximum values. The D'agostino test was applied to analyze the normal distribution of data. Statistical differences among the groups presenting normal distribution were evaluated by Unpaired *t* test. The correlation analysis between the methods was performed by Spearman's test. All analyses were performed by the Graph Pad Prism Version 6.0 software. The significance level established was 5%.

RESULTS

Patient Demographic and Clinical Data

No statistical difference was observed when comparing the percentage of each sex within the groups of patients with liver diseases. However, it is worth noting that there was a predominance of males (64.4%) among the patients with HCV (Table 1). HCV positive patients also presented a higher age average (54.8 years, $p < 0.01$) compared to the schistosomiasis group. No statistical difference was observed between analyzed groups when compared to the average BMI.

Table 1. Demographic and clinical data of patients with liver diseases (HCV or schistosomiasis).

Variables	HCV (n = 45)	Schistosomiasis (n = 17)	p-value
Sex n (%)			
Male	29 (64.4%)	08 (47.1%)	*0.21
Female	16 (35.6%)	09 (52.9%)	
Age (mean ± SD)	54.8 ± 10.2	48.5 ±18.7	**0.002
Minimum – Maximum	32 - 79	16 - 84	
BMI (mean ± SD)	24.4 ± 3.9	25.1 ± 4.2	**0.56
Minimum – Maximum	15.2 - 34.7	16.9 - 32	
Hepatic Cirrhosis n (%)	02 (4.4%)	--	

HCV = hepatitis C virus; SD = Standard Deviation; BMI = Body Mass Index. *Fisher exact test. ** Unpaired *t* test.

Biochemical marker data in patients with liver diseases

HCV positive patients showed high levels of AST (59.2 ± 43.4 U/L) when compared to schistosomiasis patients (35.5 ± 17.5 U/L, $p < 0.05$; Figure 1A). Similar results were also observed in ALT levels between the study groups. Patients with HCV presented ALT values (74.9 ± 71.2 U/L) higher than those in the schistosomiasis group (37.4 ± 16.1 U/L); however no statistical difference was reached ($p = 0.12$, Figure 1B).

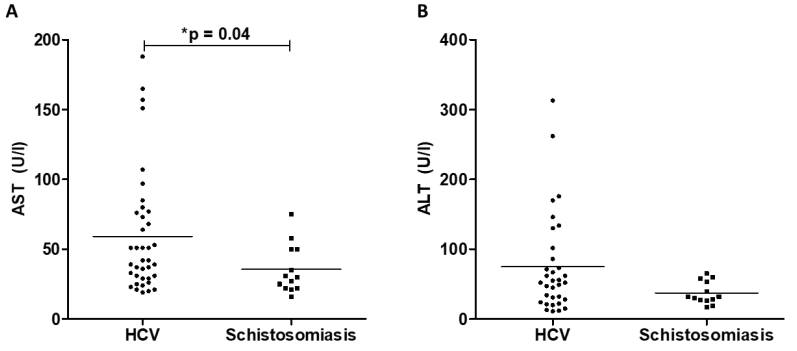

Figure 1. Serum levels of liver transaminases according to the sex of the patients with liver HCV or schistosomiasis infection. A) Aspartate Aminotransferase AST and B) Alanine Aminotransferase-ALT. Statistical differences among the groups presenting normal distribution were evaluated by Unpaired *t* test.

Correlation analysis between the methods (APRI, FIB-4 and Elastography)

A correlation analysis was performed, by Spearman test (r), to check the level of agreement among the three noninvasive methods to classify the degree of hepatic fibrosis of patients in the study groups. Both a positive and significant correlation was observed between the methods of APRI and FIB-4 in patients of the examined groups: HCV (Spearman $r = 0.79$ and $p < 0.0001$) and schistosomiasis (Spearman $r = 0.87$ and $p < 0.0001$), (Figure 2A-B).

Positive and significant correlation was also observed between the methods APRI and Transient Elastography in patients with HCV (Spearman $r = 0.67$ and $p < 0.0001$) (Figure 2C). However, a significant correlation was not observed for patients with schistosomiasis (Spearman $r = 0.50$ and $p = 0.11$) (Figure 2D).

In the correlation analysis between FIB-4 and Transient Elastography, positive correlation was observed between the groups of patients with HCV (Spearman $r = 0.50$ and $p < 0.01$) and with schistosomiasis (Spearman $r = 0.70$ and $p = 0.01$) (Figure 2E-F).

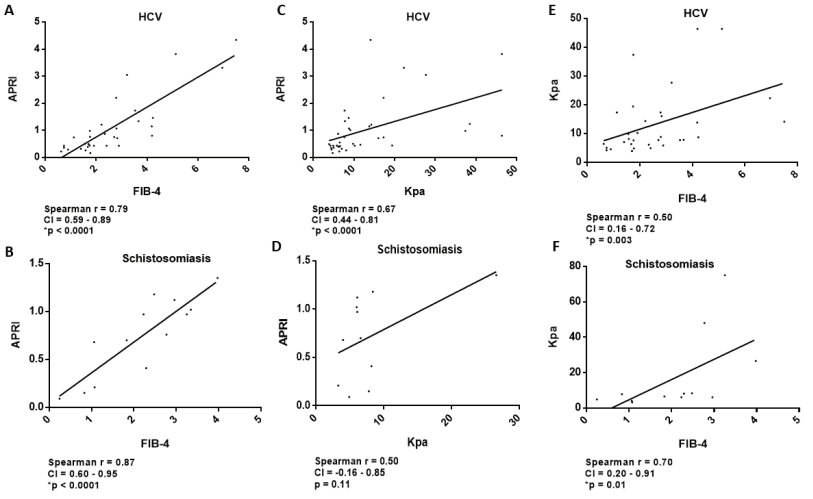

Figure 2. Correlation charts between APRI and FIB-4 by Spearman test (r) in patients carrying A) HCV and B) Schistosomiasis. Correlation charts between APRI and Transient Elastography (Kpa) by Spearman's test (r) in patients carrying C) HCV and D) Schistosomiasis. Correlation charts between Transient Elastography (Kpa) and FIB-4 by Spearman's test (r) in patients carrying E) HCV and F) Schistosomiasis.

Since the enzyme γGT can be high even in low levels of subclinical liver dysfunction (Pratt, 2016), a correlation analysis was conducted between the serum levels of γGT and the values of Kpa. There was a significant positive correlation in the HCV group (Spearman $r = 0.47$ and $p < 0.01$) and schistosomiasis (Spearman $r = 0.68$ and $p < 0.01$) (Figure 3A-B). In addition, a correlation analysis was performed between platelet counts and the values of Kpa. An inverse correlation was observed in the group of patients with HCV (Spearman $r = - 0.41$ and $p < 0.01$) (Figure 3C). A significant correlation was not observed in patients with schistosomiasis (Spearman $r = - 0.06$ and $p = 0.83$) (Figure 3D).

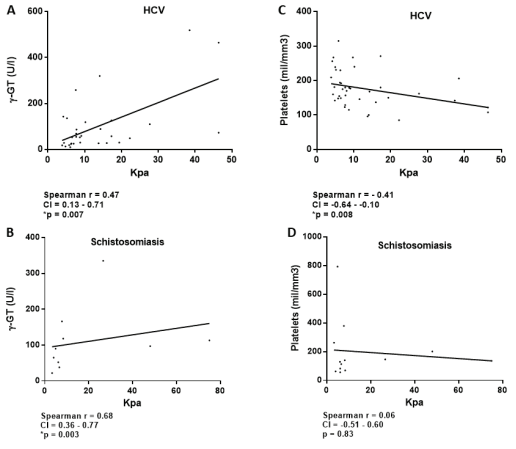

Figure 3. Correlation charts between γGT and Transient Elastography (Kpa) by Spearman's test (r) in patients carrying A) HCV and B) Schistosomiasis. Correlation charts between Platelets and Elastography (Kpa) by Spearman's test (r) in patients carrying C) HCV and D) Schistosomiasis.

DISCUSSION

The accurate assessment of the degree of fibrosis is relevant to the treatment and prognosis of liver diseases. Techniques presenting accuracy, reproducibility, and that do not put the patient at risk are required in methods for monitoring hepatic fibrosis. In this study, we analyzed the degree of agreement among three non-invasive methods for classification of hepatic fibrosis in patients with viral hepatitis and schistosomiasis. There was a strong correlation between the scores APRI and FIB-4 in all groups, regardless of the liver disease studied. This is easily explained by the fact that these scores share the same biochemical parameters, diverging only in relation to ALT and age which are part of the calculation of the FIB-4, and which are not necessary for calculating APRI. The differences between sex, mean age and BMI seem to have influenced this result.

This study showed a correlation between the results of hepatic transient elastography, APRI and FIB-4 in patients with chronic hepatitis C. These three methods have been independently validated as good non-invasive evaluators to determine the degrees of hepatic fibrosis in this pathology (Castera et al., 2005; Ferrenci et al., 2014; Schiavon et al., 2014; Veiga et al., 2017). The evaluation of the correlation between methods with different principles, however, enables the increase of result reliability in clinical practice, especially in cases with intermediate degrees of fibrosis, where these methods, used individually, may have limitations. Castera et al. (2005) showed that in a study with 183 patients, the AUROCs of association between the transient elastography and the APRI

were better than when analyzed individually, especially for F2 and F3 patients where both methods are less accurate. In another study, Veiga et al. (2017) analyzed the ultrasonographic parameters and compared them with transient elastography in hepatosplenic schistosomiasis (HES) and hepatitis C virus-cirrhotic patients. They found that patients with HES presented significantly higher liver stiffness than the controls and lower than the cirrhotic patients. The authors concluded that liver stiffness may be a useful tool to differentiate portal hypertension related to cirrhosis from that of HES.

On examining the patients with schistosomiasis, an agreement between the results of the transient elastography and the FIB-4 was noted. There was a correlation tendency between the transient elastography and APRI. However, we reviewed a small sample, therefore further studies are required with a larger sample for more accurate conclusions.

The association between the results of transient elastography and levels of gamma glutamyl transferase range (γGT) in the group of patients with chronic hepatitis C. Everhart & Wright (2013) showed that the activity of gamma-GT in the beginning was associated with the degree of fibrosis and the presence of cirrhosis, as well as the predicted fibrosis progression, despite the fact that the change in fibrosis score is not associated with the alteration in the γGT value, and patients with this high marker had a faster fibrosis progression rate. Another study showed that early readings of high γGT are an independent predictor for the occurrence of hepatocellular carcinoma, particularly in patients with cirrhosis (Huang et al., 2014). The existence of this correlation could mean that the joint assessment of these two parameters is useful in making decisions regarding treatment and prognosis of the disease.

A correlation between the results of transient elastography and gamma-GT in schistosomiasis patients was also noted. However, works on this topic are scarce in the literature and we would need a larger sample to determine if this correlation can be used clinically.

In view of the various non-invasive methods to estimate the degree of liver fibrosis, liver transient elastography is an innovative test, easy to perform and reproducible, but with limitations for intermediate degrees of fibrosis (Cheng et al., 2015; Chon et al., 2012; Lee et al., 2010; Lurie et al., 2015; Vergniol et al., 2011). Associating the various non-invasive methods can lead to greater accuracy in estimating the degree of fibrosis in these patients, especially in patients with the hepatitis C virus, making clinical procedures safer. The data regarding schistosomiasis patients evidenced a correlation among these methods. However, it is clear that a more accurate follow-up study is required. In conclusion, the data from this study suggest that non-invasive methods for diagnosis and monitoring of hepatic fibrosis present a high level of agreement, especially in patients with hepatitis C.

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