Thrombocytopenia as a marker of liver steatosis in a low-endemic area for schistosomiasis mansoni

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Summary

Introduction: Thrombocytopenia is commonly found in patients living in highly endemic areas for *Schistosoma mansoni*. Recently, different degrees of liver steatosis have also been associated with low platelet counts worldwide. We investigated the association of platelet counts with hepatosplenic schistosomiasis and with liver steatosis in an area of low prevalence of schistosomiasis in Brazil.
Method: Pains, a city in the state of Minas Gerais, Brazil, had a population of 8,307 inhabitants and a schistosomiasis prevalence of 8%. Four micro-areas comprising 1,045 inhabitants were selected for this study. Blood sample was collected and a complete blood count (CBC) was performed. Eighty-seven (87) patients had low platelet counts (group 1 – 8.3%) and 94 volunteers presenting normal CBC were randomized (group 2 – 8.9%). They underwent clinical and ultrasound examinations. Liver steatosis was determined as either present or absent using abdominal ultrasound. A spleen > 12 cm in length, measured by ultrasound (US), was considered to be increased. Data collected were analyzed using SPSS software version 19.0.
Results: Twenty-two patients (22/25.3%) in group 1 had liver steatosis compared with 11 volunteers (11.7%) in group 2 (p=0.02). Hepatosplenic schistosomiasis was diagnosed in two patients (p>0.05).
Conclusion: Thrombocytopenia was not a good marker of hepatosplenic schistosomiasis mansoni in a low prevalence area in Brazil. Liver steatosis was associated with thrombocytopenia in our study.

Keywords: fatty liver, *Schistosoma mansoni*, thrombocytopenia.

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Introduction

It has been estimated that 230 million people worldwide are infected with *Schistosoma* spp., with an additional 779 million at risk of infection. Currently, 2 to 6 million individuals are considered infected in Brazil.[1]

Most of the infected individuals are asymptomatic, but 5 to 10% develop periportal liver fibrosis with portal hypertension and splenomegaly. Pathologically dense bands of fibrosis around the portal tract are typical of *Schistosoma mansoni*.[2]

Schistosomal periportal fibrosis is usually assessed using imaging methods, and abdominal ultrasonography (US) has become the imaging technique of choice.[3] However, the identification of other non-invasive, inexpensive, and simple routine laboratory tests for use as surrogate markers is of interest.[4-6]

Recently, thrombocytopenia has been shown to identify hepatosplenomegaly in *Schistosoma*-endemic areas and is a promising marker of hepatosplenic schistosomiasis mansoni. Drummond et al., working in an area of moder-

ate to high endemicity, showed that thrombocytopenia (platelets < 143,000/mm^3) separated individuals with and without hepatosplenic schistosomiasis.[6]

Liver steatosis (also called fatty liver) refers to the abnormal retention of lipids within a cell. Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide with prevalence estimates ranging from 25 to 45% in most studies.[7,8] It covers a wide spectrum of hepatic disorders including plain steatosis and steatohepatitis (steatosis with inflammation) that can progress to liver fibrosis and cirrhosis.[9,10]

In the present study, we investigated the association of thrombocytopenia with hepatosplenic schistosomiasis and liver steatosis in an area of low prevalence of schistosomiasis in the Southeast of Brazil.

Method

This is a cross-sectional study carried out from July to November 2014, including residents of a lowly endemic area for *Schistosoma mansoni*.

Study location

Pains is a city with 8,307 inhabitants, distant 217 km from Belo Horizonte, capital of the state of Minas Gerais, Brazil. It has a total area of 418 km^2, with a population density of 19 people/km^2 and human development index (HDI) of 0.783.[11]

During the period of data collection, prevalence of schistosomiasis in this area was at 8% based on two quantitative stool examinations. All inhabitants from four micro-regions, namely Alvorada, Vila Crispin, Matinha and Posto Agropecuário, totaling 1,637 individuals aged 9-92 years, were invited to participate in our study.

Study population

After signing an informed consent, a complete blood count (CBC) of 1,045 participants were performed. Eighty-seven (87) patients had low platelet counts (group 1 – 8.3%) and 94 randomly chosen volunteers had normal CBC (group 2 – 8.9%). Information on demographics, clinical examination and the results of the Kato-Katz stool examination technique[12] were stored in a data bank for data analysis using SPSS 19.0 software.

Clinical examination

The 181 participants underwent anamnesis and physical examination carried out by one of the authors (JRL). Particular attention was given to the abdominal examination, specifically the right hepatic lobe, which was examined along the anterior axillary line, and the left hepatic lobe, examined along a line passing through the xiphoid process. The spleen was palpated and measured under the left costal margin with the patient in the dorsal decubitus position during deep inspiration.

Diagnosis of hepatosplenic schistosomiasis

The diagnosis was based on the following criteria: clinical evidence (hepatomegaly and splenomegaly) and ultrasound showing characteristic periportal fibrosis of the liver.

Ultrasound

All participants underwent abdominal US examination using a portable Medison Sonoace 1500 system with a 3.5-MHz probe (Samsung, Korea), being examined according to the protocol proposed by the World Health Organization (WHO) for US assessment of *Schistosoma*-related morbidity.[13] In our study, we used a spleen size cut-off point of > 12 cm (longitudinal diameter).

Liver steatosis

US evaluation of fatty liver typically consists of a qualitative visual assessment of hepatic echogenicity, measurements of the difference between the liver and kidney in echo amplitude, evaluation of echo penetration into the deep portion of the liver, and determination of the clarity of blood vessel structures in the liver (Figure 1A). Steatosis was characterized as present or absent.[14,15]

Ethical considerations

This study was approved by the Human Research Ethical Board of the Federal University of São João del-Rei and of the Faculty of Medicine of the Federal University of Minas Gerais (number 856.022).

Statistical analysis

Questionnaire data and results of physical exams and US were transferred into an EpiData database, software version 3.1 (EpiDataAssociation, Odense, Denmark), and analyzed using the Statistical Package for Social Sciences (SPSS) 19.0 (SPSS, IBM Company, Chicago, IL). Categorical variables were compared using $\chi 2$ Pearson test. Whenever variables in the univariate analysis presented $p<0.20$ they were included in the multivariate logistic regression analysis to evaluate the association with thrombocytopenia < 143,000/mm^3. Multivariate logistic regression was also performed to identify the independent association of significant variables with the presence of liver steatosis.

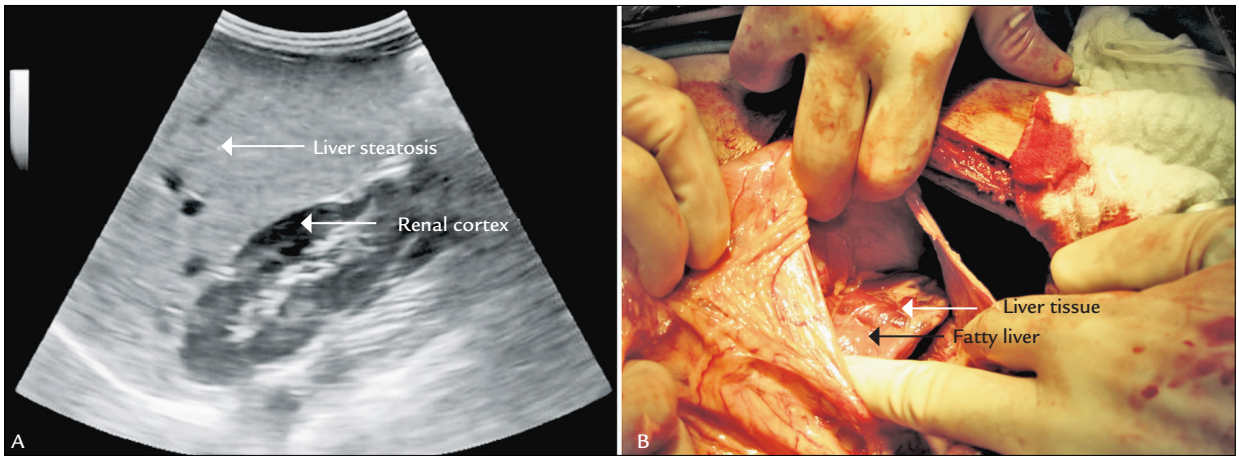

FIGURE 1 A. Ultrasonography of liver steatosis. B. Hepatosplenic schistosomiasis and liver steatosis.

Results

The demographic and clinical data of the participants are summarized in Table 1. All variables in the univariate analysis with p<0.20 were included in the model and age, gender, skin color, body mass index (BMI), general health status and liver steatosis were selected for further analysis. In the multivariate analysis, except for age, no significant correlation was detected, but a high frequency of liver steatosis called attention and we decided to investigate the association with other variables.

The association of liver steatosis with thrombocytopenia and BMI was found significant in the multivariate logistic regression (Table 2).

Discussion

Our study revealed a significant association of liver steatosis with body mass index (BMI) (p<0.01) and thrombocytopenia (p<0.02). Unexpectedly, no correlation was found between thrombocytopenia and hepatosplenic schistosomiasis in this lowly endemic area for *Schistosoma*. In previous studies, thrombocytopenia was a good marker of schistosomiasis in highly endemic areas.[5,7] This opens new ways of approaching the subject. NAFLD was associated with thrombocytopenia presently and has been described previously.[16,17] Additionally, the association of steatosis with schistosomiasis may aggravate liver disease, increasing the frequency of fibrosis, portal hypertension and liver cirrhosis.

In areas of high standard of living, the relation between thrombocytopenia and liver steatosis is a possible explanation for the finding of low platelet counts in the blood. In a lowly endemic area for schistosomiasis, as in the present study, only 2 out of 181 (1.1%) individuals had typical periportal fibrosis caused by schistosomiasis and portal hypertension.

With the rising prevalence of obesity and metabolic disorders, liver steatosis has become a leading cause of chronic liver disease in Western countries. For example, steatosis is described in 46% of heavy drinkers, 40-69% of patients with diabetes and in 50-80% of the obese population.[18,19] In Brazil, obesity is also a serious public health problem. However, social classes are not homogeneously distributed in the country: there is an increasing group of high and middle class people in the southeast, and smaller areas of low income people in the northeast of Brazil.[20]

Pains is a city located in a high- and middle-income area and most people were well nourished (35.9% of the population was overweight or obese) by the time of our study, while in the northeast of Minas Gerais the prevalence of overweight is low. The medical, educational and social assistance was superior in Pains than those offered in other poorer areas. Moreover, the Brazilian Program for Schistosomiasis Control has been active in the last 10 years in this city.

A number of studies demonstrated an association between platelet counts and the severity of liver injury.[21-25] Yoneda et al. and Ruiz-Arguelles et al. concluded that NAFLD should be considered as a cause of thrombocytopenia.[16,17]

Normally, the spleen stores one-third of the platelets that are produced in the body, maintaining a balance with the circulating platelets. Patients with cirrhosis, schistosomiasis, portal hypertension or splenomegaly may have significant degrees of "apparent" thrombocytopenia (with or without leukopenia and anemia), but they rarely have clinical bleeding, since their total platelet mass is usually normal.

Whether the presence of schistosomal periportal fibrosis is associated with NAFLD would be an interesting topic for further study. In our hospital, one patient underwent surgical intervention for treatment of portal

TABLE 1 Demographic and clinical variables of groups 1 (with thrombocytopenia) and 2 (without thrombocytopenia) in Brazil from July to November 2014.

Demographic variables	Thrombocytopenia		p-value*
	No (n=94) Group 2	Yes (n=87) Group 1	
Genre			
Male	64 (68.1%)	48 (55.2%)	0.074
Age			
Median	22.0	53.0	<0.001
Color			
White	34 (36.2%)	46 (52.9%)	
Black	27 (28.7%)	11 (12.6%)	0.078
Dark-skinned	33 (34%)	25 (28.7%)	
Use of alcohol			
Yes	26 (27.7%)	23 (26.4%)	0.823
No	65 (69.1%)	62 (71.3%)	
Clinical variables	**Thrombocytopenia**		**p-value***
	No (n=94) Group 2	**Yes (n=87) Group 1**	
General state			
Good	92 (97.9%)	76 (87.4%)	0.019
Regular	1 (1.1%)	10 (11.5%)	
Body mass index (BMI)			
Median	22.1	24.8	<0.001
Hepatosplenic schistosomiasis			
Yes	1 (1.0%)	1 (1.1%)	
No	93 (98.9%)	86 (98.8%)	0.954
Liver steatosis			
Yes	11 (11.7%)	22 (25.3%)	
No	83 (88.3%)	65 (74.7%)	0.021
Gastrointestinal bleeding			
Yes	6 (6.4%)	7 (8.0%)	0.679
No	86 (91.5%)	79 (90.8%)	
Platelets			
Median	218,000	123,000	0.958

Logistic regression $\chi 2$ Pearson test.

TABLE 2 Association between the independent variables body mass index (BMI) and thrombocytopenia and the presence of liver steatosis. Pains, state of Minas Gerais/Brazil from July to November 2014.

Independent variables	Odds ratio (95CI)	p-value*
BMI	1.21 (1.2-1.8)	<0.001
Thrombocytopenia < 143,000/mm^3	16.66 (1.4-93.2)	0.024

*Multivariate logistic regression.

hypertension, and a photograph documented the association during the surgical procedure (Figure 1B).

The association of thrombocytopenia with hepatosplenic schistosomiasis motivated our study. Our intention was to investigate the soundness of using thrombocytopenia as a marker of hepatosplenic schistosomiasis. In the end, thrombocytopenia was, in fact, associated with NAFLD. A possible explanation for disagreement is that the association of thrombocytopenia with hepatosplenic schistosomiasis previously occurred in highly endemic areas,[6] while, presently, our investigation was performed in an area of low prevalence (8%) and low morbidity (two patients with hepatosplenic schistosomiasis). We conclude that thrombocytopenia is a good marker of liver steatosis in this lowly endemic area for schistosomiasis. The association of schistosomiasis with thrombocytopenia was not confirmed in the present study.

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Conflict of interest

The authors declare no conflict of interest.

Resumo

Trombocitopenia como marcador de esteatose hepática em áreas de baixa endemicidade de esquistossomose mansoni

Introdução: Trombocitopenia é um achado comum em pacientes que residem em áreas com alta endemicidade de esquistossomose mansônica. Recentemente, diferentes graus de esteatose hepática também têm sido associados a níveis baixos de plaquetas em todo o mundo. Investigamos a associação de níveis séricos de plaquetas com a forma grave da esquistossomose e com esteatose hepática em área de baixa prevalência de esquistossomose no Brasil.
Método: Pains, cidade localizada no estado de Minas Gerais/Brasil, tem população de 8.307 habitantes e prevalência de esquistossomose de 8%. Em quatro microáreas dessa região, 1.045 habitantes foram avaliados para o estudo. Amostra de sangue foi coletada para realização do hemograma. Oitenta e sete (87) pessoas com níveis baixos de plaquetas formaram o grupo 1 (8,3%), e 94 voluntários com hemograma normal foram randomizados para compor o grupo 2 (8,9%). Todos os participantes dos grupos 1 e 2 foram submetidos a exame clínico e ultrassonografia (US) abdominal. Esteatose hepática foi caracterizada como presente ou ausente pela ultrassonografia (US) abdominal. Baços com mais de 12 cm de comprimento à US foram considerados aumentados. Os dados coletados foram analisados pelo programa de estatística SPSS 19.0.
Resultados: Vinte e dois (22) indivíduos do grupo 1 (25,3%) e 11 do grupo 2 apresentaram esteatose hepática (11,7%) (p=0,02). Esquistossomose hepatoesplênica foi diagnosticada em dois pacientes (p>0,05).
Conclusão: Trombocitopenia não foi um bom marcador de esquistossomose mansônica hepatoesplênica em área de baixa prevalência da esquistossomose no Brasil. Esteatose hepática foi associada com trombocitopenia no presente estudo.

Palavras-chave: esteatose hepática, *Schistosoma mansoni*, trombocitopenia.

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