UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
PÓS-GRADUAÇÃO EM BIOQUÍMICA E FISIOLOGIA

TESE DE DOUTORADO

DISFUNÇÕES DA COAGULAÇÃO E DA FIBRINÓLISE EM PACIENTES COM ESQUISTOSSOMOSE MANSÔNICA NA FORMA HEPATOESPLÊNICA

LUIZ ARTHUR CALHEIROS LEITE

RECIFE
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Tese apresentada ao Programa de Pós-graduação em Bioquímica e Fisiologia da Universidade Federal de Pernambuco, como requisito para obtenção do grau de Doutor em Bioquímica.

RECIFE
2014

Catalogação na Fonte:
Bibliotecário Bruno Márcio Gouveia, CRB-4/1788

Leite, Luiz Arthur Calheiros.
Disfunções da coagulação e da fibrionólise em pacientes com esquistossomose mansônica na forma hepatoesplênica / Luiz Arthur Calheiros Leite. – Recife: O Autor, 2014.

109 folhas: il.
Orientadores: Vera Lúcia de Menezes, Ana Lúcia Coutinho Domingues, Edmundo Lopes de Almeida Pessoa.
Tese (doutorado) – Universidade Federal de Pernambuco. Centro de Ciências Biológicas. Bacharelado em Ciências Biológicas, 2014.
Inclui bibliografia e anexos

1. Esquistossomose 2. Sangue – Coagulação I. Menezes, Vera Lúcia de (orient.) II. Domingues, Ana Lúcia Coutinho (coorient.) III. Pessoa, Edmundo Lopes de Almeida III. Título.

616.963 CDD (22.ed.) UFPE/CCB-2014-141

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Data 14/02/2014.

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2014

Dedico este trabalho a Deus, aos meus familiares e aos amigos.

AGRADECIMENTOS

Primeiramente a Deus, pela minha existência e por tudo que já conquistei até aqui.

A minha família, sobretudo a meu pai e à minha mãe, Antônio Jessé Leite e Anaci Calheiros Leite, pelo incentivo e apoio em todos os momentos. A meus irmãos, Cynthia e Ricardo, e a minha filha Maria Alice Pugliesi Leite, motivo pelo qual realizei este trabalho e pretendo seguir a carreira de pesquisador.

À Profa. Dra. Vera Lúcia de Menezes Lima, por ter acreditado no meu potencial e pelas oportunidades que me proporcionou; principalmente, por não ter desistido da orientação, sempre me ensinando o real papel de um pesquisador: ser humilde, ético e sensato.

À Profa. Dra. Ana Lúcia Coutinho Domingues, pelo apoio, pelas frequentes revisões e pelo incentivo constante oferecido integralmente durante todo o trabalho. Por ter permitido o acompanhamento dos pacientes esquistossomóticos na realização dos exames ultrassonográficos e viabilizado a realização das coletas nos pacientes com esquistossomose no serviço de ultrassonografia do setor de Gastroenterologia do Hospital das Clínicas de Pernambuco – UFPE.

Ao prof. Dr. Edmundo Lopes, pelo constante incentivo, pela revisão dos artigos e por nunca ter me deixado desanimar, sempre me mostrando que em pesquisa devemos ter muita paciência. Por ser um entusiasta e por me receber ora como um professor, ora como um pai.

À profa. Dra. Silvia Montenegro, pela disponibilidade para a realização dos testes de fibrinólise no Centro de Pesquisa Aggeu Magalhães – FIOCRUZ-PE.

Aos meus mais que colegas de Laboratório: À professora Vera Cristina de Oliveira Carvalho, Adenor, Caíque, Bianka, Priscila, Tiago, Janaína, Tiago Ferreira, Cleideana, Dewson, Weber, Myza, José, Shalon, Ana Paula, Irailton, Ilton, João, Marilia e a Rita de Cassia Fereira, Clara, Liana Macedo, que sempre me ajudaram com os dados clínicos da pesquisa.

À equipe da Unidade de Laboratório do Hospital das Clínicas de Pernambuco UFPE, na pessoa de Jacqueline Martins, pela colaboração.

A todos os professores e demais funcionários do Programa de Pós-Graduação em Bioquímica e Fisiologia, em especial aos senhores João Virgílio, Albérico Real, Djalma Gomes da Silva, Ademar e Fredson.

Aos colegas da Fundação Hemope, principalmente a Betânia e Ana, do Laboratório de Hemostasia.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), à Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo apoio financeiro.

E por último, aos participantes mais importantes desta pesquisa, os pacientes esquistossomóticos, que foram sempre simpáticos e em nenhum momento negaram qualquer informação ou assinatura, mesmo convivendo com esta doença negligenciada e de alta morbidade.

“A educação é a arma mais forte que se tem para mudar o mundo.”

Nelson Mandela

“A imaginação é mais importante que o conhecimento.”

Albert Einstein

“Quase todos os homens são capazes de suportar adversidades, mas se quiser pôr à prova o caráter de um homem, dê-lhe poder.”

Abraham Lincoln

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LISTA DE ABREVIATURAS

Anti-HCV – Anticorpo antivírus da hepatite C

ALT: Alanina aminotransferase

AST: Aspartato aminotransferase

FA: Fosfatase alcalina

γGT: gama-glutamil transferase

HBag: Antígeno de superfície do vírus da hepatite B

HBV: Vírus da hepatite B

HCV: Vírus da hepatite C

HE: Forma hepatoesplênica

PAI-1: Plasminogen activator inhibitors 1

OMS: Organização Mundial de Saúde

t-PA: Tissue type plasminogen activator

TP: Tempo de protrombina

TTPa: Tempo de tromboplastina parcial ativada

TT: Tempo de trombina

RESUMO

A esquistossomose hepatoesplênica (HE) é a forma mais grave desta doença, sendo caracterizada por fibrose periportal (FPP), hipertensão portal, esplenomegalia e citopenias. Pacientes com esquistossomose na forma HE podem apresentar disfunções hemostáticas que predispõem a tromboses e hemorragias. Este estudo teve como objetivo avaliar as alterações hepáticas e da coagulação em 55 pacientes com esquistossomose na forma HE, 45 esplenectomizados, 30 pacientes com DHCM, bem como em 30 indivíduos normais acompanhados no Serviço de Gastroenterologia do Hospital das Clínicas da Universidade Federal de Pernambuco. As provas de função hepática [aminotransferases (AST e ALT), fosfatase alcalina (FA), gama-glutamil transferase (γ-GT), albumina, bilirrubinas totais e frações] foram determinadas por métodos cinéticos, e os testes de coagulação, tempo de protrombina (INR), tempo de tromboplastina parcial (PTT), fatores da coagulação (II, VII, VIII, IX, X), proteína C, antitrombina IIa e D-dímero foram mensurados por método cromogênico. Os ensaios fibrinolíticos [ativador do plasminogênio tissular (t-PA), inibidor-1 do ativador de plasminogênio 1 (PAI-1)] foram realizados por ELISA. Os resultados mostraram que os pacientes com a forma HE exibiram níveis mais elevados de AST, ALT, γ-GT, FA, INR, PTT, fibrinogênio, D-dímero e diminuição da albumina, fatores VII, IX, X e proteína C, quando comparados com os indivíduos controles ($p<0,0001$). Em relação aos diferentes grupos com padrões de fibrose, verificou-se apenas que a concentração da proteína C apresentou diferença entre os padrões D e E+F ($p = 0,01$). Os pacientes esplenectomizados exibiram níveis menores de FA ($p=0,03$), maiores de bilirrubinas ($p=0,04$) e albumina ($p=0,02$), além de diminuição do INR, PTT, fatores VII, IX, X e proteína C quando comparados com os pacientes HE, ($p<0,001$). Estes achados evidenciam que as disfunções hemostáticas estão relacionadas com a fibrose periportal avançada, hipertensão portal, e que a proteína C é capaz de estratificar os diferentes padrões de fibrose. A esplenectomia, além de reduzir a pressão portal por diminuir o fluxo na veia porta, também promoveu melhora no *status* funcional hepático e hemostático dos pacientes com esquistossomose mansônica.

Palavras-chaves: Esquistossomose, Coagulação, Fibrose periportal, Hipertensão portal.

ABSTRACT

The hepatosplenic form (HS) is the most severe form of schistosomiasis, characterized by periportal fibrosis (PPF), portal hypertension, splenomegaly and cytopenias. The purpose of this study was to evaluate the liver and coagulation tests in 55 HS patients, 45 splenectomized, 30 mixed disease and 30 normal controls. The diagnosed was perfomed by ultrasound examinaton and all patients were followed at the Department of Gastroenterology, Hospital das Clinicas, Federal University of Pernambuco. The liver function tests [aminotransferases (AST and ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT), albumin, bilirubins] were determined by cinetic methods, and coagulation tests, prothrombin index (INR), partial thromboplastin time (PTT), coagulation factors (II, VII, VIII, IX, X), protein C, antithrombin IIa and D-dimer were measured by chromogenic technique. The fibrinolytic parameters [tissue plasminogen activator (t-PA), inhibitor-1 plasminogen activator 1 (PAI- 1)] were performed by ELISA. The results showed that HS patients exhibited higher levels of AST, ALT, γ- GT, ALP, INR, PTT, fibrinogen, D-dimer and decreased of albumin, factors VII, IX, X and protein C when compared to health controls. According to patterns of PPF, just protein C levels had significantly decreased between the patterns D and E+F ($p=0.01$). Splenectomized patients showed lower levels of ALP ($p=0.03$), increase of bilirubin and albumin, and decrease of INR, PTT, factors VII, IX and X when compared to HS patients ($p<0.001$). These findings show that haemostatic dysfunction are related with advanced patterns of PPF can leads to a progressive liver damage and impairment of blood coagulation proteins, and that protein C is able to stratify different patterns of PPF. Furthermore, splenectomy remains a therapeutic effective in reducing portal flow and improves the hemostatic status of patients with schistosomiasis.

Keywords: Schistosomiasis, Blood coagulation, Periportal fibrosis, Portal hypertension.

1. INTRODUÇÃO

A esquistossomose é uma doença parasitária negligenciada, que afeta cerca de 240 milhões de pessoas em todo o mundo, sendo considerado um sério problema de saúde pública (GRYSEELS et al., 2012). Pode ser causada por espécies diferentes, dentre estas se destaca a esquistossomose mansônica, comum em regiões tropicais e subtropicais, tais como, o continente africano, Caribe e Brasil, com alta prevalência na região Nordeste e no estado de Minas Gerais (ROSS et al., 2002, FERREIRA et al., 2014). A forma mais grave da *S.mansoni* é conhecida como forma hepato-esplênica (HE), e aproximadamente 10% dos pacientes com esquistossomose desenvolve esta forma, que gera alta morbidade, devido à fibrose periportal avançada, hipertensão portal, esplenomegalia, citopenias e aos episódios de hemorragia digestiva (MAIA et al, 2007; DISCH et al, 2002; BARBOSA et al., 1996).

As anormalidades nos testes da coagulação e fibrinólise na esquistossomose têm passado despercebidas por muitos anos, contudo os distúrbios hemostáticos em pacientes com esquistossomose na forma HE podem estar relacionados a desequilíbrios entre os fatores procoagulantes, anticoagulantes e fibrinolíticos (TANABE, 2003; EL-BAUSSIONI et al., 1996). Dentre estas anormalidades, detacando-se o prolongamento do tempo de protrombina bem como a trombocitopenia e a hipofibrinogenemia. Além disso, estudos têm mostrado que a redução dos fatores dependentes de vitamina K (II, VIII, IX e X) e as alterações no sistema fibrinolítico podem aumentar o risco de hemorragias em pacientes com esquistossomose. Estas anormalidades têm sido constantemente atribuídas à hipertensão portal e à disfunção hepática precoce, muitas vezes não detectada pelos testes de função hepáticas usuais (EL-BAUSSIONI et al., 1996; FERRAZ et al., 2001, OMRAN et al.,1994).

Paradoxalmente, muitos pacientes com esquistossomose mansônica na forma HE, apresentam normalidade nos valores das provas de função hepática, mesmo em indivíduos com fibrose periportal avançada ou muito avançada (CAMACHO-LOBATO&BORGES, 1998). Contudo, as enzimas canaliculares tais como a γ-glutamil-transferase e a fosfatase alcalina bem como as proteínas da

coagulação, tais como o fator VII e a proteína C sofrem alteração nos seus níveis séricos, indicando que pode existir diminuição na capacidade de síntese hepática e que os marcadores usuais devem possuir baixa sensibilidade para detectar estas alterações. Além disso, o aumento destas enzimas e proteínas pode sinalizar disfunção hepática precoce, pois os níveis de aminotransferases permanecem normais e a hipertensão portal pode ser um fator adjuvante no processo de dano hepático e canalicular. Já a proteína C da coagulação, pode ser estratificar diferentes padrões de fibrose periportal, além de ser considerado o principal marcador para detectar a etiologia da trombose de veia porta (CAMACHO-LOBATO&BORGES, 1998; LEITE et al., 1996),

Este estudo teve por objetivo analisar as alterações nas proteínas e enzimas hepáticas, da coagulação e fibrinolíticas em pacientes com esquistossomose mansônica na forma HE e nos pacientes com e sem baço, tentando relacionar esses achados com os diferentes padrões de fibrose periportal e com a hipertensão portal.

2. FUNDAMENTAÇÃO TEÓRICA

A esquistossomose é endêmica em vários países e acomete cerca de 240 milhões de pessoas, com 120 milhões de casos com manifestações clínicas e 20 milhões de formas graves. Trata-se de uma doença endêmica na África (*Schistosoma mansoni, S. haematobium e S. intercalatum*), na Venezuela, Caribe e Brasil (*S. mansoni*), na China, Indonésia e Filipinas (*S. japonicum*) (ROSS et al., 2002; GRYSEELS et al., 2012).

No Brasil, estima-se que existam de 2,5 milhões a 6 milhões de infectados pelo *S. mansoni*, principalmente nos Estados de Minas Gerais, Rio Grande do Norte, Paraíba, Pernambuco, Bahia, Alagoas e Sergipe. No Nordeste, 90% dos pacientes infectados apresentam formas leves e moderadas, e cerca de 3 a 5% evoluem para a forma grave da doença, a forma HE (DISCH et al., 2002; PORDEUS et al., 2008; SILVA et al., 2002).

Com a chegada dos vermeis e ovos aos ramos terminais à veia porta, inicia-se um processo inflamatório granulomatoso limitado com posterior estímulo para a formação de fibrose periportal e obstrução pré-sinusoidal. Contudo a fibrose periportal ou fibrose de Symmers permanece persiste como marca do processo inflamatório e parasitário. Essa alteração é uma característica marcante e essencial para o diagnóstico e a diferenciação da esquistossomose de outras doenças hepáticas que cursam com fibrose. (ANDRADE, 2009; GRYSEELS et al., 2006).

O processo fibrótico e a obstrução pré-sinusoidal leva a alterações no fluxo portal, devido à formação de uma camada de colágeno nos ramos portais e consequente aumento da pressão porta e esplênica. Este hiperfluxo portal, gera várias alterações, tais como a esplenomegalia, e o hiperesplenismo que, frequentemente resulta em trombocitopenia, leucopenia e podendo o paciente exibir até pancitopenia. O sequestro esplênico pode intensificar os episódios de hemorragia digestiva (hematemase e melena) e contribuir com o surgimento de equimoses em alguns pacientes com esquistossomose na forma HE (LEITE et al, 2013, Montenegro, 2013, MAIA et al, 2009).

A hipertensão portal é causada pelo bloqueio no fluxo portal intra-hepático, decorrente da formação dos granulomas e da fibrose em torno dos ramos portais,

como também pelo hiperfluxo proveniente do baço, devido à esplenomegalia. Essas lesões granulomatosas e fibróticas são responsáveis por alterações vasculares intra-hepáticas. Em decorrência da hipertensão portal surgem varizes gastroesofágicas, que predispõem à hemorragia digestiva alta. Além disso, estudos clínicos tem mostrado que a redução dos fatores de coagulação aumenta o risco de sangramento por ruptura das varizes esofágicas. Estas anormalidades são mais notáveis nas fases nas avançadas da esquistossomose e nos que exibem padraõ de fibrose periportal avançada (MAIA et al., 2007; TANABE, 2003).

Entretanto, muitos achados relacionados à hepatopatia esquistossomótica permanecem incertos, pois pacientes com esquistossomose na forma HE exibem diferentes padrões de fibrose, mesmo com o parênquima hepático conservado e os testes de função hepática normais, seja em pacientes com fibrose periportal avançada ou mesmo com diferentes graus de hipertensão portal. A lesão hepática na esquistossomose não atinge todo o parênquima hepático justificando a normalidade das aminotransferases, que estão localizadas dentro dos hepatócitos. Além disso, a esquistossomose não leva a destruição de hepatócitos, e sim a diminuição de síntese hepática, associada à fibrose e a hiperfluxo portal e esplênico. Com isso, há diminuição das proteínas da coagulação (II, VII, IX, X, PC) e aumento da γ-GT e FA, associada ao dano endotelial secundário a hipertensão portal (ANDRADE, 2009; LEITE et al., 2013).

Os pacientes com esquistossomose na forma HE apresentam-se geralmente em estado compensado. A figura 1 mostra um paciente que se apresenta com hepatomegalia, principalmente do lobo esquerdo do fígado e esplenomegalia. Este paciente não apresentava sinais clínicos de descompensação, tais como ascite, icterícia ou mesmo encefalopatia. A esplenomegalia resulta de estímulos imunológicos desencadeados pelos antígenos dos ovos do *S. mansoni*, promovendo hiperfluxo para a veia porta e maior aumento da pressão. A esplenomegalia pode ainda levar ao hiperesplenismo, decorrente de maior proliferação do sistema monocítico macrofágico fagocítico, resultando em citopenias periféricas (anemia, leucopenia e trombocitopenia) secundárias ao sequestro esplênico (ANDRADE, 2009; GRYSEELS et al., 2006).

Com a progressão da doença, 10% destes pacientes evoluem para formas descompensadas, com presença de ascite, coagulopatia, icterícia, sinais de encefalopatia hepática, albumina abaixo de 3 g/dL, perda de massa muscular e ginecomastia. Essa descompensação é mais comum em pacientes esquistossomóticos que apresentam hemorragia digestiva ou em pacientes com doença hepática mista (CAMACHO-LOBATO, BORGES, 1998; OMRAN et al., 1994).

A esplenectomia com desconexão ázigo-portal ou associada à ligadura da veia gástrica esquerda com ou sem ligadura intraesofagiana das varizes do esôfago tem se mostrado eficaz na prevenção de recidivas de hemorragia digestiva por reduzir a pressão portal. Todavia, a escolha deste tipo de procedimento cirúrgico ainda provoca controvérsias, uma vez que nenhuma intervenção se mostrou plenamente eficaz e isenta de efeitos adversos, a exemplo do aumento da frequência de trombose de veia porta (EVANGELISTA-NETO et al., 2012; FERRAZ et al., 2001).

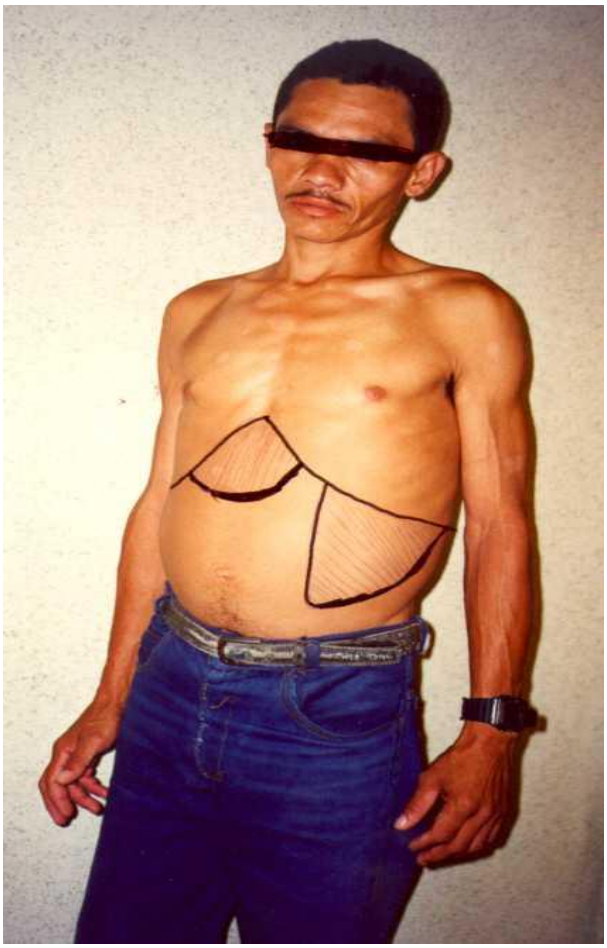

Figura 1 – Paciente com Esquistossomose mansônica na forma hepatoesplênica. Fonte: Cedido pela professora Doutora Ana Lúcia Coutinho Domingues-Gastroenterologia, Hopsital das Clínicas, UFPE, (2013).

2.1 Aspectos clínicos da esquistossomose mansônica

A hepatomegalia reflete a presença de inflamação granulomatosa e ocorre antes da evolução para a forma HE. A deposição de colágeno periportal leva a uma obstrução progressiva do fluxo sanguíneo intra-hepático na veia porta, hipertensão portal, esplenomegalia e hiperesplenismo, varizes de esôfago e sangramento digestivo, sendo o hiperesplesnismo um marcador de hipertensão portal em formas mais graves da doença (KÖPKE-AGUIAR et al., 2002; MAIA, 2007).

A fibrose periportal pode ser visualizada através da ultrassonografia, tomografia computadorizada ou ressonância magnética. A função de síntese

hepática é preservada em pacientes com esquistossomose compensados, fato que por diversas vezes diferencia a hepatopatia esquistossomótica de pacientes cirróticos (GRYSEELS et al., 2006; ANDRADE, 2004).

A descompensação em pacientes esquistossomóticos também pode estar relacionada com as manifestações secundárias à hipertensão portal, como o hiperfluxo portal e esplênico, disfunção endotelial, desenvolvimento de varizes gastroesofágicas, hemorragias digestivas, hipotensão arterial, isquemia dos hepatócitos e desregulação imunológica. Todo esse conjunto de fenômenos, somados à fibrose periportal, pode levar à lesão hepatocelular com deposição de colágeno e alterações vasculares na arquitetura do tecido hepático. Essas mudanças são importantes para que a esquistossomose na forma HE possa ser considerada uma doença hepática vascular. Os pacientes descompensados frequentemente apresentam albumina baixa, encefatopatia, ascite, aumento do lodo esquerdo, esplenomegalia mais volumosa e falência hepática, podendo esta relacionado com desnutrição, idade elevada e ingestão de álcool (GRYSEELS et al., 2006; ANDRADE, 2004).

2.2 Classificações da fibrose periportal

Por muitos anos, o diagnóstico de esquistossomoese e, principalmente da forma HE, era realizado por palpação abdominal e identificação do aumento do fígado e do baço. Contudo, nas últimas três décadas, o exame ultrassonográfico tem se tornado a melhor e mais utilizada ferramenta para avaliação das alterações provocadas pelo *S.mansoni* no fígado, no baço e nos ramos portais. Com a finalidade de padronizar as lesões fibróticas no fígado, a OMS reuniu no Cairo vários pesquisadores que elaboraram a Classificação do Cairo (The Cairo Working Group, 1992). A fibrose periportal foi classificada em graus I a III. Devido às discordâncias na diferenciação dos casos sem fibrose e fibrose grau I, esta classificação foi revista em Niamey, Sudão, sendo criada a Classificação de Niamey. A Classificação de Niamey definiu os padrões de fibrose de A a F, comparando pranchas

preestabelecidas em que A significa ausência de fibrose, B fibrose duvidosa, C fibrose periférica, D fibrose central, E fibrose avançada e F fibrose muito avançada (RITCHER et al., 2001). A figura 2 mostra os padrões da fibrose periportal que acomete os pacientes com esquistossomose na forma HE. Estes pacientes exibem padrões de fibrose entre D e F. No padrão de fibrose central – D, a fibrose periportal é bem mais localizada quando comparada com a fibrose avançada – E e muito avançada - F que se estende pelo parênquima hepático Na esquistossomose HE é importante também medir o diâmetro da veia porta, o tamanho do lobo esquerdo e direito do fígado, o diâmetro longitudinal do baço e veia esplênica, o espessamento da vesicular biliar, bem como caracterizar os diversos tipos de circulações colaterais presentes nestes pacientes (RITCHER et al., 2001).

Essa técnica tem como vantagem a economia e a simplicidade do método de avaliação dos órgãos abdominais e pode ser realizada utilizando dispositivos portáteis que permitem a sua aplicação em regiões endêmicas. Porém, técnicas complementares são necessárias para mensurar o risco de hemorragia digestiva alta e o calibre das varizes gastroesofágicas (finas, médias e grossas), sendo detectadas e classificadas pela endoscopia digestiva. Esse procedimento permite também tratar as varizes com escleroterapia e ligadura elástica, e assim diminuir ou mesmo evitar recidiva de sangramentos digestivos (DIAS et al., 2013). A esplenomegalia é destacada e mensurada pela ultrassonografia, e é definida através de um valor de corte, pois quando o baço apresenta-se maior que 12 cm (esplenomegalia), e exibem aumento do lodo direito, diminuição do esquerdo e fibrose periportal, classifica-se o paciente como hepato-esplênico (RITCHER et al., 2001).

Além disso, a ultrassonografia possibilita distinguir casos de cirrose hepática da esquistossomose na forma HE e de outras hepatopatias cirrogênicas, como ilustrado na figura 3 que mostra a fibrose difusa pelo parênquima hepático e a fibrose periportal (AQUINO et al., 2000; RITCHER et al., 2001; CAMACHO-LOBATO, BORGES, 1998).

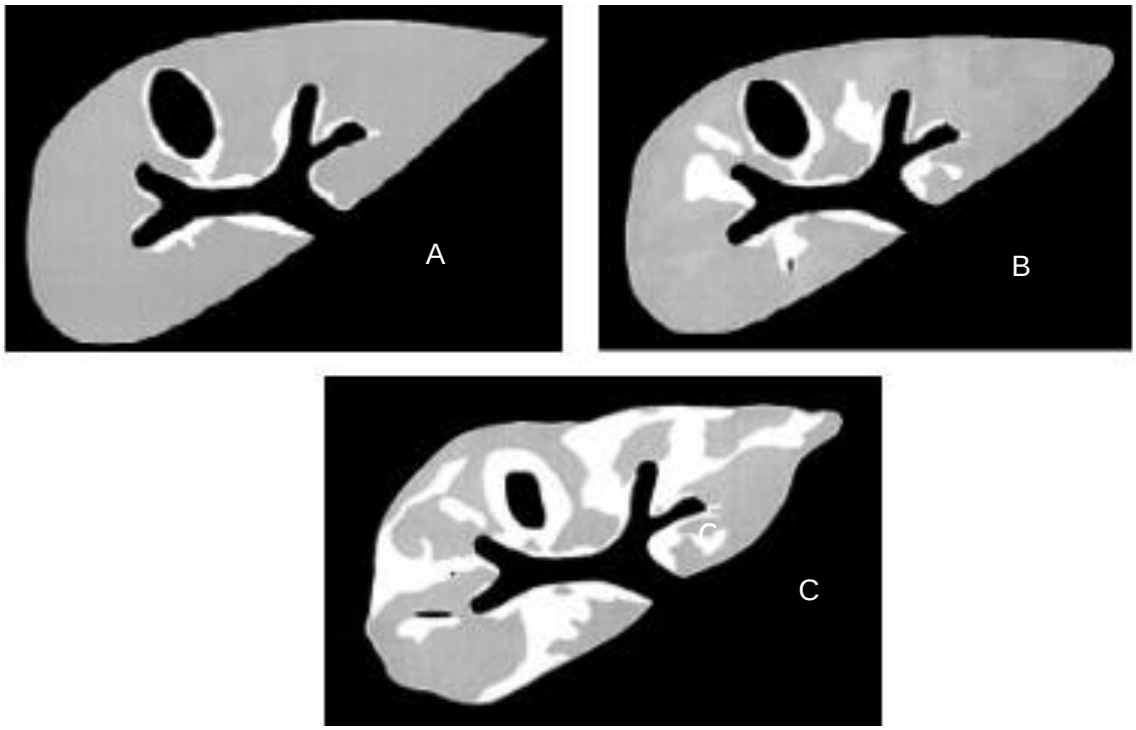

Figura 2 – Diferentes padrões de fibrose periportal:

A – Padrão D (fibrose periportal central), B – Padrão E (fibrose periportal avançada com manchas ecogênicas e expansão dentro do parênquima), C − Padrão F (fibrose periportal muito avançada com espessamento periférico periportal). Fonte RITCHER et al., 2001.

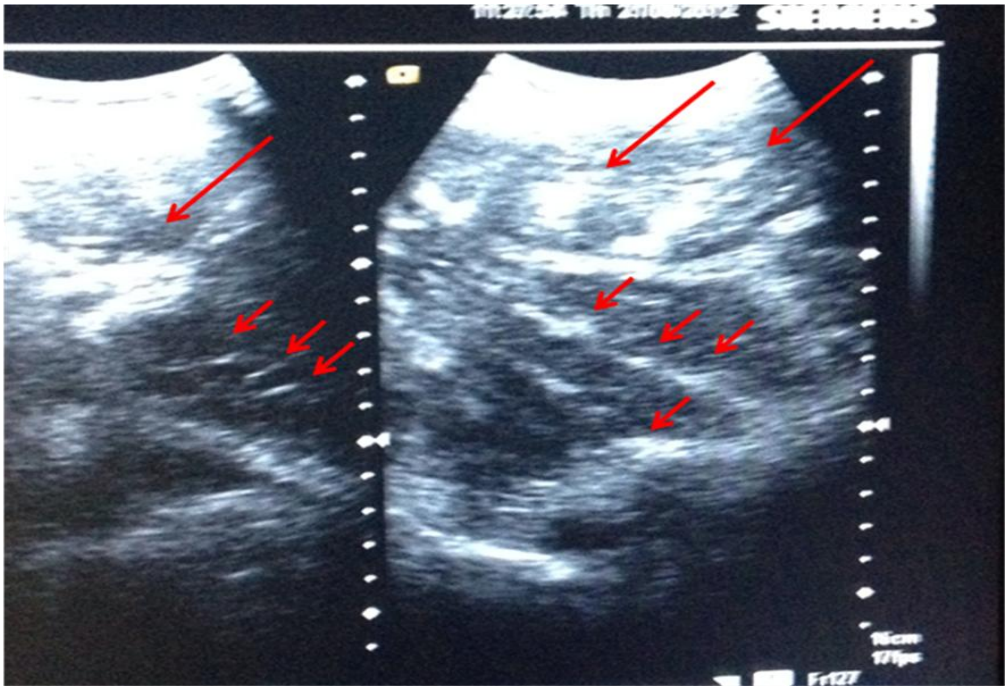

Figura 3 – Imagem de ultrassonografia do abdômen com doença hepática crônica mista (fibrose fina difusa pelo parênquima − setas pequenas − e fibrose periportal − setas grandes). Fonte: Fonte: Cedido pela professora Doutora Ana Lúcia Coutinho Domingues-Gastroenterologia, Hopsital das Clínicas, UFPE (2013).

2.3 Alterações da função hepática na esquistossomose

Os pacientes com esquistossomose na forma HE apresentam sinais de alterações hepáticas resultantes da fibrose periportal e hipertensão portal em intensidades diferentes da cirrose, visto que alguns pacientes com esquistossomose exibem função hepática preservada e nem todos descompensam. Portanto, a principal diferença clínica entre a esquistossomose na forma HE e a cirrose é o comprometimento hepático, comum em pacientes cirrótico e muitas vezes ausente em pacientes com esquistossomose (ANDRADE, 2004; CAMACHO-LOBATO & BORGES, 1998).

Como os hepatócitos contêm inúmeras enzimas no citoplasma e na mitocôndria, em virtude do dano hepático pode ocorrer liberação dessas enzimas no plasma, sendo úteis no monitoramento das disfunções hepáticas. Dentro do

citoplasma está contida a alanina aminotransferase (ALT), e na matriz mitocondrial encontra-se grande concentração de aspartato aminotransferase (AST), que são liberadas mediante a lesão mitocondrial, comumente em hepatopatias crônicas avançadas. Na maioria dos pacientes com esquistossomose na forma HE, as aminotransferases encontram-se em níveis plasmáticos normais ou minimamente elevados, provavelmente devido a ausência de lesão mitocondrial dos hepatócitos (DOMINGUES et al., 2011).

Muitos trabalhos tem mostrado em pacientes com hipertensão portal cirrótica, a dependência da função hepática ao fluxo sanguíneo hepático e ao fluxo portal. Esses trabalhos evidenciaram interferência importante do fluxo sanguíneo portal na atividade funcional do fígado. É conhecido, entretanto, que a hipertensão portal cirrótica é localizada nos sinusóides, hipertensão portal sinusoidal, e que se associa à lesão hepatocelular importante. Deste modo, a função das células está comprometida e estas se tornam mais lábeis às mudanças de sua perfusão. Já os pacientes com hipertensão portal por esquistossomose têm geralmente a arquitetura lobular e a função hepática mantidas, sendo as alterações vasculares hepáticas e do sistema porta os aspectos implicados na fisiopatogenia da hipertensão portal esquistossomótica (ALVES Jr. et al., 2003; AMARAL et al., 2002). Com isso, a manutenção dos níveis das enzimas hepáticas normais (AST e ALT) em pacientes esquistossomóticos provavelmente tem relação com a manutenção da perfusão hepática por vencer o bloqueio pré-sinusoidal e preservar a função do fígado (ALVES Jr. et al., 2003; AMARAL et al., 2002).

Já as enzimas canaliculares como a fosfatase alcalina (FA) e a γ-glutamil transferase (γGT) estão elevadas no plasma em pacientes esquistossomóticos com obstruções pré-canaliculares. Estudos têm mostrado que a γGT se eleva nos pacientes com esquistossomose na forma HE, principalmente nos pacientes que apresentam padrões de fibrose periportal avançada. Os mecanismos propostos para explicar a elevação da γGT bem como da FA estão relacionados às alterações hepatobiliares (ALVES Jr. et al., 2003; AMARAL et al., 2002). As elevações dessas enzimas e o possível comprometimento hepático em pacientes com esquistossomose têm origem predominantemente vascular. Atribuem-se as

alterações nos níveis da γGT bem como da FA à colestase e à hipertensão portal, principalmente nas formas mais avançadas da doença (ALVES Jr. et al., 2003). Assim, a γGT, pode ser mais sensível que a FA para detectar alterações colestáticas em pacientes com esquistossomose e o dano hepático observado em alguns pacientes com esquistossomose na forma HE ocorre por maior comprometimento hepático por maior congestão e por alterações puramente vasculares (ALVES Jr. et al., 2003; AMARAL et al., 2002).

É possível que cirurgias que possam diminuir a hipertensão portal e as anormalidades hemodinânicas, tais como a esplenectomia sejam capazes de diminuir a congestão hepática e restaurar a capacidade de síntese dos hepatócitos em pacientes com esquistossomose na forma HE. Contudo tal possibilidade necessita de comprovação cientifica através de novas investigações com diferentes metodologias ratificar esta hipótese (ALVES Jr. et al., 2003).

CAMACHO-LOBATO & BORGES, 1998 demostraram que existe alterações hepáticas precoces nos pacientes com esquistossomose na forma hepatoesplênica, com altos níveis de AST, ALT, γGT e FA quando comparados com pacientes que apresentavam a forma hepatointestinal. Mesmo assim, este achado permanece controversio, pois de acordo com a teoria do hepatócito intacto, a função hepática nas doenças hepáticas crônicas esta associada com redução do número de hepatócitos e raramente a diminuição da função hepática. Além disso, outros estudos relatam preservação da função hepática em pacientes com esquistossomose normais, com valores de AST, ALT normais (LEITE et al., 2013).

Entretanto, existe ainda um grande desafio no diagnóstico das hepatopatias, pois a quantidade de marcadores plasmáticos é pequena e muitos possuem especificidade e sensibilidade limitadas ou mesmo tardias, sendo necessários novos marcadores capazes de medir a função hepática de forma sensível tanto para lesão hepática como para estratificar diferentes padrões de fibrose periportal. Para tanto, as proteínas da coagulação podem ser extremamente úteis na avaliação da função hepática, por possuírem menor meia vida plasmática e terem maior sensibilidade e especificidade que a AST e a ALT em pacientes com esquistossomose (CAMACHO-LOBATO & BORGES, 1998).

2.4 Evidências de anormalidades nos testes de coagulação em pacientes com esquistossomose

O compromentimento da coagulação no curso da doença esquistossomótica tem sido amplamente relatada na literatura e vem sendo considerada uma alteração natural durante o curso da esquistossomose na forma hepatoesplênica. Estas alterações vêm sendo associadas à redução da síntese hepática dos fatores da coagulação ou menor depuração das proteínas plasmáticas que compõem o sistema hemostático, diminuição dos níveis dos inibidores, tais como a proteína C, e mudanças nos componentes do sistema fibrinolítico. Além disso, esta disfunção pode ser atribuída ao consumo dos fatores plasmáticos das proteínas da coagulação, entretanto, as evidências de coagulopatia de consumo ainda permanecem sem explicação, podendo ser subclínica ou em baixo grau e, portanto pode ocasionar alterações clinicas tais como tromboses em apenas alguns pacientes com esquistossomose (TANABE, 2003; CAMACHO-LOBATO, BORGES, 1998; OMRAN et al.,1994, LEITE et al., 2013).

Contudo estes mecanisco permanecem incertos (Estes achados são um extremamente paradoxal, pois é amplamente aceito que pacientes com esquistossomose mesmo na forma hepatoesplênica exibem preservação da função hepática mesmo em fases tardias da doença, com níveis de albumina normais contrastando com o prolongamento do TP, PTTA e TT e hipofibrinogenemia. Nesses casos, as proteínas de coagulação (fatores II, VII, IX, X e proteína C) mostram redução em suas atividades, o que se torna mais notável em pacientes esquistossomóticos com fibrose periportal avançada, pois exibem níveis mais baixos de proteína C (TANABE, 2003; CAMACHO-LOBATO, BORGES, 1998; OMRAN et al.,1994, LEITE et al., 2013).

A redução significativa de proteínas de coagulação pode contribuir para o surgimento de hemorragias digestivas nos pacientes infectados com *S. mansoni*, sobretudo nos pacientes com esquistossomose na forma hepatoesplênica com

fibrose avançada e que exibem elevação dos níveis de γGT, fato que contribui para a redução progressiva da síntese hepática dos fatores II, VII, IX, X e da proteína C. Essa disfunção também resulta na diminuição dos níveis de proteína C e no aumento do fator VIII, com maior predisposição à trombose, sendo a proteína C um marcador precoce de disfunção hepática, principalmente quando associado γGT, fostatase alcalina e ao fator VII, pois tanto o fator VII quanto a proteína C possuem meia vida plasmática curta (TANABE, 2003; CAMACHO-LOBATO&BORGES, 1998; OMRAN et al., 1994, LEITE et al.,2013).

Os números de plaquetas comumente estão diminuídas em pacientes com esquistossome na forma hepatoesplênica. Além disso, outras anormalidades hematológicas podem ser vistas em pacientes com esquistossomose, tais como leucopenia, bicitopenias, pancitopenias e mais raramente anemia por perda sanguínea. Estes achados podem ser atribuídos a esplenomegalia e ao hiperesplenismo que leva a maior captura de plaquetas e leucócitos pelos macrófagos do baço. Além disso, a diminuição da contagem de plaquetas pode esta relacionada com a coagulopatia de consumo. Kopke-Aguiar, 2009 mostraram que pacientes com esquistossomose na forma hepatoesplênica possuem níveis normais de trombopoietina, levando a crer que não existe alteração da trombopoeise e de megacariócitos nestes pacientes e que a redução do número de plaquetas possui relação direta com a esplenomegalia (LEITE et al, 2013; MAIA et al., 2007; CAMACHO-LOBATO&BORGES, 1998).

CORREIA et al., 2009, mostraram que pacientes com esquistossomose na forma HE apresentam níveis elevados de fator de von Willebrand, podendo ser considerado um fator de proteção contra hemorragias, por aumentar a adesão e a agregação das plaquetas em pacientes com trombocitopenia leve ou moderada. MAIA et al., 2007, evidenciaram que quanto maior o tamanho do baço, menor a contagem de plaquetas, o que pode levar a plaquetopenias graves com manifestações hemorrágicas.

Estudos de grupos do Egito têm mostrado significantes alterações nas proteínas e enzimas fibrinolíticas em pacientes com esquistossomose na forma HE descompensados, como também aumento dos níveis de D-dímero, t-PA e redução

do PAI-1, principalmente em pacientes com padrões de fibrose avançada e trombocitopenia associada (OMRAN et al., 1990; EL-BAUSSIONI et al., 1996). O prejuízo na função hepática pode ocasionar menor depuração do t-PA, resultando em maior risco de sangramento para os pacientes com esquistossomose na forma hepatoesplênica. A redução da depuração hepática pode estar associada ao desvio da circulação hepática. Além disso, a presença de circulações colaterais nos pacientes esquistossomóticos possui correlação com níveis elevados de t-PA, mostrando que ocorre menor passagem desta enzima pelo fígado e consequentemente menor depuração (CAMACHO-LOBATO&BORGES, 1998; LEITE et al., 2013).

Em um recente estudo do nosso grupo foi demonstrado que alguns pacientes com esquistossomose na forma hepato-esplênica com fibrose avançada exibem níveis de D-dimero acima de 483 ng/mL, e que quando somados a redução dos níveis de PAI-1, e elevação do t-PA, levam a um status de hiperfibrinólise e maior risco hemorrágico, principalmente quando associados a hipertensão portal (LEITE et al., 2013; OMRAN et al., 1990; EL-BAUSSIONI et al., 1996).

As anormalidades nas proteínas da coagulação e da fibrinólise em pacientes com esquistossomose na forma HE não são relacionados apenas com a disfunção hepática, mas também às alterações vasculares e hemodinâmicas. Esses achados reforçam que a diminuição do fluxo portal através da esplenectomia resulta em significativa melhora nos níveis das proteínas da coagulação (aumento concomitante dos fatores VII, IX, X, proteína C e de algumas provas de função hepática, como FA, albumina e bilirrubina total e frações), e consequente diminuição do risco de sangramento em pacientes com esquistossomose na forma HE, mesmo em formas avançadas da doença. A esplenectomia leva também ao aumento dos níveis de PAI-1, melhorando a capacidade de síntese dos hepatócitos e reduzindo o fluxo portal, pois o PAI-1 é sintetizado tanto pelo fígado como pelas células endoteliais (TANABE, 2003; OMRAN et al., 1990; EL-BAUSSIONI et al.,1996).

A figura 4 ilustra os componentes da coagulação, anticoagulação e fibrinólise, assim como o mecanismo de ativação e inibição da hemostasia em pacientes com esquistossomose na forma hepatoesplênica. Nesse esquema representativo, estão

indicados os riscos de trombose e sangramentos de acordo com o desequilíbrio entre elevações ou reduções dos níveis das proteínas da coagulação e fibrinólise em pacientes com esquistossomose na forma HE. A figura também demonstra que a diminuição de qualquer um dos fatores dependentes de vitamina K (II, VII, IX, X) pode levar a sangramentos, principalmente quando associado à redução no número de plaquetas. Além disso, os níveis de proteína C devem ser monitorizados com frequência, pois qualquer redução desde leve a intensa nos níveis plasmáticos desta proteína, pode ocasionar trombose de veia porta, esplênica ou mensentérica. A figura também destaca que em pacientes com esquistossomose na forma hepatoesplênica possuem alterações da fibrinólise, como aumento dos níveis de D-dimero, t-PA e diminuição do PAI-1, achados que pode aumentar o risco de sangramento, independente das demais proteínas da coagulação. O D-dimero também pode ser utilizado como marcador precoc de tromboses. Vale destacar que as disfunções hemostáticas na esquistossomose podem ser discretas ou mesmo precoces, devido à relativa preservação da síntese hepática, (CAMACHO-LOBATO, BORGES, 1998).

Conclui-se que o aumento da geração de trombina e plasmina em pacientes com esquistossomose pode indicar *status* de coagulação intravascular disseminada subclínica, fato que, associado ao dano hepático, pode ser responsável pela disfunção hemostática. O *status* de coagulopatia subclínica pode aumentar também o risco de sangramento desses pacientes, devido ao consumo dos fatores de coagulação. Com isso, qualquer desequilíbrio entre os fatores da coagulação e os níveis de proteína C pode resultar em tromboses, e quando somados ao *status* hiperfibrinolítico, aumentam de forma significativa o risco hemorrágico em pacientes com esquistossomose na forma HE (TANABE, 2003; CAMACHO-LOBATO, BORGES, 1998).

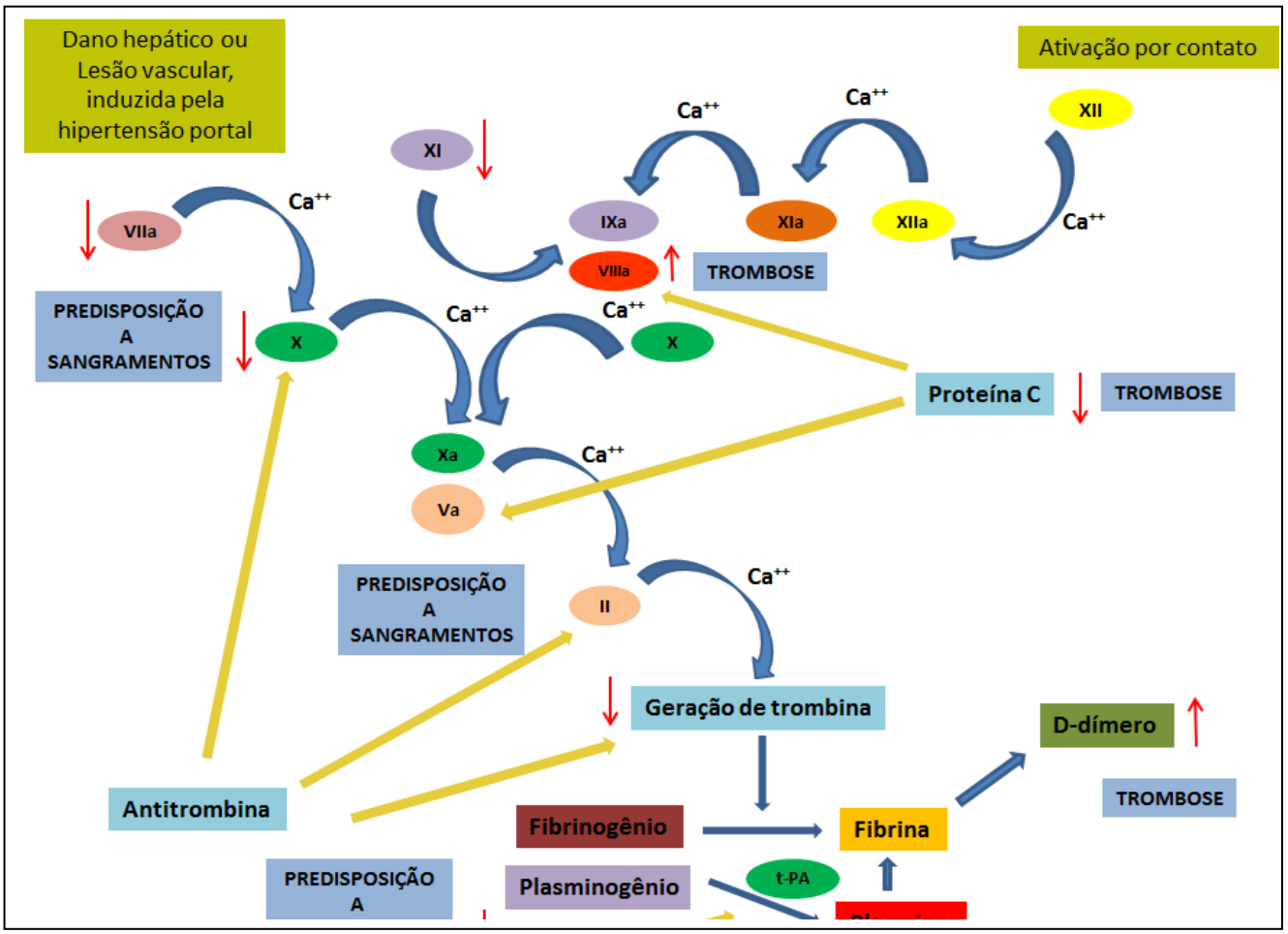

Figura 4 - Representação da cascata de coagulação, efeito anticoagulante e relação com o sistema fibrinolítico em pacientes com esquistossomose na forma HE. As setas em azul representam ativação e a setas em amarelo, efeito hemostático inibitório. O sentido para baixo das setas em vermelho indica redução das proteínas envolvidas no sistema hemostático e o sentido para cima indica o aumento destas proteínas. Os quadros em azul são indicativos de tendência à trombótica ou hemorrágica em pacientes com esquistossomose na forma HE ou mesmo com doença hepática mista. Fonte: O Autor (2013).

3. JUSTIFICATIVA

Pacientes com esquistossomose mansônica podem evoluir para complicações clínicas e, portanto, passam a apresentar mal prognóstico. Uma dessas complicações é a alteração do sistema de coagulação e da fibrinólise, que pode estar relacionada ao dano hepático, hipertensão portal, episódios de hemorragia digestiva. Tanto as alterações hemorrágicas como as trombóticas podem acometer muitos pacientes com esquistossomose, constituindo um conjunto de anormalidades que merece atenção especial tanto em nível clínico como laboratorial. Para tanto, este estudo teve mostrou que uma investigação minuciosa dos testes de coagulação e da fibrinólise são extremamente necessários, visto que muitos pacientes com esquistossomose apresentam disfunções hemostáticas subclínicas que podem aumentar a morbidade desta doença. Além disso, os testes de cogulação podem ser utilizados como provas de função hepática, pois possuem maior sensibilidade a lesões canaliculares e hepática, devido a curta meia vida.

4. OBJETIVOS

4. 1. GERAL:

Avaliar as alterações da coagulação e da fibrinólise em pacientes com esquistossomose mansônica na forma hepatoesplênica, em pacientes submetidos à esplenectomia.

4. 2. ESPECÍFICOS:

1. Investigar a ocorrência de disfunções hemostáticas em pacientes com esquistossomose na forma HE em diferentes padrões de fibrose periportal.
2. Verificar a influência da esplenectomia sobre os testes hemostáticos em pacientes esquistossomóticos com e sem o baço.
3. Estudar os níveis das protéinas e enzimas fibrinolíticas nos pacientes com esquistossomose na forma HE, nos pacientes com e sem o baço.

REFERÊNCIAS

ALVES JR A, FONTES DA, MELO VA, MACHADO MCC, CRUZ JF, SANTOS EAS. Hipertensão Portal Esquistossomótica: influência do fluxo sanguíneo portal nos níveis séricos das enzimas hepaticas. **Arq Gastroenterol**. v. 40, p. 203-208, 2003.

AMARAL ACC, KÖPKE-AGUIAR LA, SOUZA MRA, TOLEDO CF, BORGES DR. Elevação da γ-glutamiltransferase sérica na hepatopatia esquistossomótica não se correlaciona com a carga parasitária e precede alterações ultrassonográficas. **Arq Grastroenterol**. v. 39, p. 27-31, 2002.

ANDRADE, ZA. Schistosomiasis and liver fibrosis. **Parasite Immunology**. v.31, p. 656-663, 2009.

ANDRADE, ZA. Schistosomal hepatopathy. **Mem. Inst. Oswaldo Cruz**. v. 95, p 51-57, 2004.

BARBOSA, CS; SILVA, CB; BARBOSA, FS. Esquistossomose: Reprodução e expansão da endemia no estado de Pernambuco no Brasil. **Revista de Saúde Pública**. v. 30, p. 609-616, 1996.

CAMACHO-LOBATO L, BORGES DR. Early liver dysfunction in schistosomiasis. **Journal of Hepatology**. v 29, p. 233-240, 1998.

CORREIA MCB, DOMINGUES ALC, LACERDA HR, SANTOS EM, MACHADO CGF, HORA V, NEVES MA, BRITO A, CÔELHO RCB, SILVA JLA. Platelet function and the von Willebrand factor antigen in the hepatosplenic form of schistosomiasis

mansoni. **The Royal Society of Tropical Medicine and Hygiene** v. 103, p. 1053-1058, 2009.

DIAS HS; DOMINGUES ALC; CORDEIRO FTM; JUCÁ N, LOPES EPA. Associating portal congestive gastropathy and hepatic fibrosis in hepatosplenic mansoni schistosomiasis. **Acta Tropica**, v. 126, p. 240-243, 2013.

DISCH J, KATZ N, SILVA YP, VIANA LG, ANDRADE MO, RABELLO A. Factors associated with schistosomiasis mansoni infection 5 years after selective treatment in low endemic area in Brazil. **Acta Tropica**, v. 81, p. 133-142, 2002.

DOMINGUES ALC; MEDEIROS TB, LOPES EPA. Ultrassound versus biological markers in the evaluation of periportal fibrosis in human schistosoma mansoni. **Mem. Inst. Oswaldo Cruz**, v. 106, p. 802-807, 2011.

EL-BASSIOUNI NE, EL-BASSIOUNY AE, EL-KRAYAT, OMRAN SA. Hyperfibrinolysis in hepatosplenic schistosomiasis. **Journal of Clinical Pathology**. v. 49, p 990-993, 1996.

EVANGELISTA-NETO J, PEREIRA FF, FRANÇA ST, AMARAL FJ, BRANDT CT, FONSECA-NETO OCL, LACERDA CM. Splenectomy and gastric vein ligature in hepatosplenic schistosomiais: effects upon esophageal variceal pressure and endoscopic risk factors of esophageal variceal bleeding. **Arquivos Brasileiros de Cirurgia Digestiva**. v. 25, 41-48, 2012.

FERRAZ AAB, ALBUQUERQUE PC, LOPES EAP, ARAUJO JR JGC, BARROS FMR, SETTE JA, ARRUDA SMB, FERRAZ EM. Esplenectomia com ligadura da veia

gástrica esquerda e desvascularização da grande curvatura do estômago no tratamento da esquistossomose e hepatoesplênnica. È necessária a escleroterapia endoscópica pós-operatória? **Arq Gastroenterol**. v. 38, p. 84-88, 2001.

GRYSEELS B, POLMAN K, CLERINX K, KESTENS L. Human schistosomiasis. **The Lancet** 368: 1106–18, 2006.

KOPKE-AGUIAR LA, MARTINS JRM, PASSEOTTI CC, TOLEDO CF, NADER HD. Serum hyaronic acid as a comprehensive marker to assess severity of liver disease in schistosomiasis. **Acta Tropica**. v. 84, p. 117-126, 2002.

LEITE LAC, PIMENTA FILHO AA, FONSECA CSM, FERREIRA RCS, DOS SANTOS BS, MONTENEGRO SLOPES EP, DOMINGUES ALC, OWEN JS, LIMA, VLM. Hemostatic Dysfunction is Increased in Patients with Hepatosplenic Schistosomiasis mansoni and Advanced Periportal Fibrosis, **Plos Neglected Diseases**. v. 18, e2314; p. 1-5, 2013.

LEITE LAC, DOMINGUES ALC, LOPES EP, FERREIRA RCP, PIMENTA FILHO AA, FONSECA CS, S, DOS SANTOS BS, LIMA, VLM. **Rev Bras Hemat Hemoter**. v. 35, p. 332-336, 2013.

MAIA MD, LOPES EPA, FERRAZ AAB, BARROS FMR, DOMINGUES ALC, FERRAZ EM. Evaluation of splenomegaly in the hepatosplenic form of mansonic schistomiasis. **Acta Tropica.** v. 101, p. 183-86, 2007.

OMRAN SA, AMIN HM, EL-BASSIOUNI NE, ESSAWY FM, TOIEMA SM. Vitamin K dependent coagulation proteins in endemic hepatosplenomegaly in Egypt. **Journal of Clinical Pathology**. v. 47, p. 502-504, 1994.

OMRAN SA, HUSSEIN NA, MOHAMED AA, EL-KALIOUBY AH, HUSSEIN AT. Fibrinolysis and bleeding tendency in patients with hepatosplenic schistomiasis. **J Clin Pathol** v. 43, p. 476-478, 1990.

PORDEUS LC, AGUIAR LC, QUININO LRM, BARBOSA CS. A ocorrência das formas agudas e crônica da esquistossomose mansônica no Brasil no período de 1997 a 2006. Uma revisão de literatura. **Epidemiol Serv. Saúde.** v 17, p 163-175, 2008.

RICHTER J, DOMINGUES ALC, BARATA CH, PRATA AR, LAMBERTUCCI JR. Report of the Second Satellite Symposium on Ultrasound in Schistosomiasis. **Mem Inst Oswaldo Cruz**. v. 96, p.151-56, 2001.

ROSS AGP, BARTLEY PB, SLEIGH AC, OLDS GR, YUESHENG LI, WILLIAMS GM, McMANUS DP. Schistosomiasis**. The New England Journal of Medicine.** v. 346, p. 1213-1220, 2002.

SILVA, SN, DE OLIVEIRA, KF, BRANDT, C. T, LIMA, VLM. Estudo dos lipídeos em jovens portadores de esquistossomose hepatoesplênica submetidos a tratamento cirúrgico. **Acta Cirúrgica Brasileira***,* v.17, p.251-7, 2002.

SILVA, JMA, SOUZA VSB, VILELLA TAS, DOMINGUES ALC, COÊLHO MRCD. Soroprevalência da hepatite C em pacientes com esquistossomose. **Arq Gastroenterol**. v. 48, p. 124-129, 2011.

TANABE M. Haemostatic abnormalities in hepatosplenic schistosomiasis mansoni. **Parasitology Internacional**. v. 52, p 351-359, 2003.

The Cairo Working Group, Ed by Jenkins JM&Hatzc. The use diangnostic ultrasound in World Health Organization (WHO). Ultrasound in schistosomiasis. A practical guide to the standardized use of ultrasonography for the assessment of schistosomiasis-related morbidity. Second International Workshop. October 22-26, Niamey, Niger, 1996.

6. RESULTADOS

CAPÍTULO I

Hemostatic Dysfunction Is Increased in Patients with Hepatosplenic Schistosomiasis Mansoni and Advanced Periportal Fibrosis

PLoS Negl Trop Dis. 2013 July; 7(7): e2314.

Published online 2013 July 18. doi: 10.1371/journal.pntd.0002314

Impact fator - 4.72

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Hemostatic Dysfunction Is Increased in Patients with Hepatosplenic Schistosomiasis Mansoni and Advanced Periportal Fibrosis

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Abstract

Background: Schistosomiasis mansoni is an endemic parasitic disease and a public health problem in Northeast Brazil. In some patients, hepatic abnormalities lead to periportal fibrosis and result in the most severe clinical form, hepatosplenic schistosomiasis. This study aimed to evaluate whether abnormal blood coagulation and liver function tests in patients with hepatosplenic schistosomiasis (n = 55) correlate with the severity of their periportal fibrosis.

Methodology/Principal Findings: Blood samples were used for liver function tests, hemogram and prothrombin time (International Normalized Ratio, INR). The blood coagulation factors (II, VII, VIII, IX and X), protein C and antithrombin IIa (ATIIa), plasminogen activator inhibitor 1 (PAI-1) and D-dimer were measured by photometry or enzyme linked immunosorbent assay. Hyperfibrinolysis was defined on the basis of PAI-1 levels and a D-dimer concentration greater than a standard cut-off of 483 ng/mL. Standard liver function tests were all abnormal in the patient group compared to healthy controls (n = 29), including raised serum transaminases ($p<0.001$) and lower levels of albumin ($p = 0.0156$). Platelet counts were 50% lower in patients, while for coagulation factors there was a 40% increase in the INR ($p<0.001$) and reduced levels of Factor VII and protein C in patients compared to the controls (both $p<0.001$). Additionally, patients with more advanced fibrosis (n = 38) had lower levels of protein C compared to those with only central fibrosis ($p = 0.0124$). The concentration of plasma PAI-1 in patients was one-third that of the control group ($p<0.001$), and D-dimer levels 2.2 times higher ($p<0.001$) with 13 of the 55 patients having levels above the cut-off.

Conclusion/Significance: This study confirms that hemostatic abnormalities are associated with reduced liver function and increased liver fibrosis. Of note was the finding that a quarter of patients with hepatosplenic schistosomiasis and advanced periportal fibrosis have hyperfibrinolysis, as judged by excessive levels of D-dimer, which may predispose them to gastrointestinal bleeding.

Citation: Leite LAC, Pimenta Filho AA, Fonseca CSMd, Santos BSd, Ferreira RdCdS, et al. (2013) Hemostatic Dysfunction Is Increased in Patients with Hepatosplenic Schistosomiasis Mansoni and Advanced Periportal Fibrosis. PLoS Negl Trop Dis 7(7): e2314. doi:10.1371/journal.pntd.0002314

Editor: Edgar M. Carvalho, Hospital Universitário, Brazil

Received December 27, 2012; **Accepted** June 3, 2013; **Published** July 18, 2013

Funding: The authors received financial support for this study from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/BRAZIL. The funders had no role in study design, data collection and analysis, in the decision to publish, or in preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Schistosomiasis is a chronic parasitic liver disease that constitutes a major public health problem in several parts of the world. There are more than 200 million people affected by schistosomiasis worldwide and 600 million people are at risk of infection [1–3]. The disease caused by *Schistosoma mansoni* is the most prevalent liver disease in the Northeast region of Brazil [4]. Around 5–7% of patients infected by *S. mansoni* progress to the most severe form, hepatosplenic. Many patients exhibit high morbidity and mortality associated with periportal fibrosis, portal hypertension and splenomegaly, which lead to frequent episodes of upper gastrointestinal bleeding [5].

Periportal fibrosis constitutes the pathognomonic lesion of the liver in hepatosplenic schistosomiasis [6–8]. This process results from massive deposition of collagen products in the periportal spaces and leads in turn to progressive occlusion of the portal vein, portal hypertension, splenomegaly, collateral venous circulation and bleeding of the upper gastrointestinal tract. GI bleeding episodes are one of the causes of hepatic dysfuction in

Author Summary

Schistosomiasis is a parasitic disease that affects the liver and in the severe hepatosplenic form results in periportal fibrosis. This disease is a major public health problem in Northeast Brazil. Our study aim was to evaluate whether abnormal blood coagulation and liver function tests in patients with hepatosplenic schistosomiasis depended on the severity of their fibrosis and could be used to inform diagnosis and treatment. We verified, by analyzing blood samples and by abdominal ultrasound of 55 patients, that blood clotting abnormalities are associated with reduced functioning of the liver and with increased liver fibrosis. Our results additionally suggested that reduced levels of protein C in plasma are a good marker of liver fibrosis progression. Also of note was our finding that a quarter of patients with advanced fibrosis have hyperfibrinolysis, a severe blood clotting disorder which may increase their risk of gastrointestinal bleeding. Therefore, we recommend, for patients eligible for surgical procedures, that certain blood tests (D-dimer, prothrombin time and platelet count) be measured during the pre-surgical evaluation to better assess risk of bleeding.

schistosomiasis; areas of hepatic necrosis can occur due to hypotension and loss of blood, which in liver regeneration can distort the hepatic parenchyma. When patients bleed more than once, hepatic dysfunction and compromised hemostasis ensue [6].

A more extensive pattern of fibrosis reflects the prognosis and severity of the chronic hepatosplenic condition [6], although it is generally reported that liver function remain preserved. On the other hand, some studies have found reduced levels of blood coagulation proteins, which are synthesized by liver cells [9–11]. Classically, it is believed that liver cell function is preserved in hepatosplenic schistosomiasis and that the compromised hemostasis is due to a consumptive coagulopathy related to the enlarged liver and spleen. In a previous study, it was suggested that early liver dysfunction in schistosomiasis may contribute to the problems with hemostasis [9]. However, it remains unclear whether an advanced pattern of fibrosis is linked to an adverse effect on hemostasis and liver function. Here, our aim was to determine whether abnormal blood coagulation and liver function tests in patients with hepatosplenic schistosomiasis correlate with the severity of their periportal fibrosis.

Materials and Methods

Ethical statement

The study was conducted according to the Helsinki Declaration and was approved by the Human Research Ethics Committee of the Federal University of Pernambuco (Number 028/11), in Brazil. All patients and healthy subjects received an explanation about the scope of the study, such as objectives, procedures and potential risks, and signed an informed consent statement before inclusion in the study.

Patients

Fifty-five patients diagnosed with hepatosplenic schistosomiasis, and previously treated with praziquantel (50 mg/Kg) at least 6 months before the present study, were the overall of those attending as outpatients between 2010 and 2012 at the Gastroenterology Department, Clinical Hospital of the Federal University of Pernambuco, Recife, Brazil. When first seen at the clinic all patients had hepatosplenomegaly and portal hypertension, but without ascites, jaundice, encephalopathy and/or pulmonary hypertension, and a history of contact with river water within municipalities located in "Zona da Mata", an endemic area for schistosomiasis in Pernambuco State. Some had reported at least one episode of upper gastrointestinal bleeding.

The diagnosis of schistosomiasis was based on clinical history, physical examination and an abdominal ultrasonography which showed periportal fibrosis. Using the World Health Organization (Niamey Working Group, 2000) protocol [12] patients were classified as having peripheral fibrosis (Pattern C), central fibrosis (Pattern D), advanced fibrosis (Pattern E) or very advanced fibrosis (Pattern F) [12,13]. Patients who presented with advanced or very advanced fibrosis were grouped together for data analyses (Pattern E+F).

Patients were excluded if they reported alcohol abuse (>60 g ethanol/day for men and >40 g/day for women) or had a history of splenectomy, hepatic cirrhosis, systemic diseases such as diabetes mellitus, acute or chronic hepatitis B or C, collagenosis, heart and blood diseases. Use of hepatotoxic drugs, acetylsalicylic acid, anticoagulant drugs, or receiving a blood transfusion were also criteria for exclusion if less than 90 days prior to data collection. The control group consisted of twenty nine healthy individuals from the same age range (18 to 65 years) and socioeconomic background, as evaluated by a standardized questionnaire that enabled family budget, education level and lifestyle to be matched with those of the patients.

Sample collection and processing

Three venous blood samples were collected under aseptic conditions without stasis using vacuum tubes (Vacutainer; *Becton Dickinson*, USA). The first tube contained 0.106M trisodium citrate at a 1:9 ratio to blood for coagulation tests. The second contained 0.562M EDTA-K3 and was used directly for platelet quantification, while the third blood collection tube was used for liver function tests. Tubes one and three were centrifuged for 10 min at 2000 *g* and the plasma and serum stored in 0.5 ml aliquots at −80°C until assayed.

Parasitological diagnosis

Stool samples from control and patient groups were taken on two consecutive days and each tested twice by the Kato-Katz method. The mean egg counts are reported.

Biochemical, hepatic and other tests

The routine liver function tests included aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), γ-glutamyltransferase (γGT), bilirubin (Total, Direct and Indirect) and albumin and were measured by automated spectrophotometry (Cobas C501, Roche, Diamond Diagnostics, USA). Determinations of HBsAg, anti-HBc and anti-HCV were made by Chemiluminescence Microparticle Immuno Assay (CeMIA) using the ARCHITECT i2000 automatic light detector and test reagents (Abbott, North Chicago, USA) to exclude enrollment of patients with Hepatitis B or C. Abdominal ultrasound avoided inclusion of patients with hepatic cirrhosis and steatosis, and the anamnesis excluded patients with active use of alcohol.

Platelet count and blood coagulation tests

Platelet counts (normal range $150–400\times10^9$/L) were measured by electrical impedance using the Pentra-120 (ABX Diagnostics, São Paulo, SP, Brazil). Coagulation tests were performed with an automated photooptical coagulometer, (Trinity Biotech, Acton, USA) and included measuring prothrombin time (PT, expressed as

the INR), partial thromboplastin time (PTT), thrombin test (TT) and fibrinogen.

Blood coagulation factors II, VII, VIII, IX, X were assayed using a Destiny Plus analyzer (Trinity Biotech, Acton, USA) and were based on correcting the long PT of factor deficient plasma by addition of test plasma diluted with clotting factor deficient plasma. Results were expressed as a percentage of activity for each factor. Protein C in test plasma was measured in the Destiny Plus analyzer with a specific snake venom protein C activator, thus inhibiting factor V and VIII in the added Protein C deficient plasma reagent and prolonging the subsequent PTT test, while antithrombin IIa was determined using saline buffer and specific reagents.

Antigenic assays to quantify tissue plasminogen activator (t-PA), plasminogen activator inhibitor −1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) in plasma were measured by sandwich enzyme-linked immunosorbent assays ELISA (Asserachrom, Diagnostica Stago, France). Each sample was tested in duplicate and measured according to the supplier's instructions. D-dimer was assayed in an automated photo-optical coagulometer; values above 483 ng/mL were defined as hyperfibrinolysis [14].

Statistical analysis

Unpaired Student's *t* test was used to compare differences between normally distributed variables of the hepatosplenic schistosomiasis patients (combined total) and control group, while the fibrosis pattern groups were compared by one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test. Mann-Whitney and Kruskal-Wallis followed by Dunn's multiple comparison tests were used to compare differences among non-normally distributed variables. Variables were expressed as mean±Standard Error of the mean. P-values less than 0.05 were considered to be statistically significant. All statistical analyses were performed using Statview SAS Inc. (1998, NC, USA).

Results

All patients presented with some degree of periportal fibrosis. The Niamey classification [12] revealed a predominance of advanced periportal fibrosis, Pattern E (n = 30; 54.6%), followed by central fibrosis, Pattern D (n = 17; 30.9%), while only 8 of the patients (14.5%) showed Pattern F, very advanced fibrosis. No patient was classified as having Pattern C (peripheral fibrosis). For easiness of data analysis, the two patterns of advanced periportal fibrosis were combined into a single group (E+F; n = 38; 69.1%).

As a total group, the patients with hepatosplenic schistosomiasis showed abnormal liver function tests compared to the healthy controls with significantly ($p<0.05$) increased levels of serum AST, ALT, γ-GT, ALP and total bilirubin, and a lower concentration of albumin (Table 1). These differences were also seen when the two patients groups (Patterns D and E+F) were compared separately with the controls, except for the albumin level in the Pattern D group which was not significantly reduced. No differences were noted when patients with central fibrosis (Pattern D) were compared to those with advanced periportal fibrosis (Pattern E+F) although the level of γ-GT was 64% higher in the latter group ($p = 0.0501$; Table 1).

The combined total groups of patients all showed significant increases in the INR, PTT and TT values compared to the healthy controls, while the platelet count ($\times 10^9$/L) was 50% lower (128±13 vs. 261±10; $p<0.001$) (Table 2). All blood coagulation factors (II, VII, VIII, IX, X and antithrombin IIa) were lower in the total patient group, and these significant differences were also seen when Pattern D and Pattern E+F were compared with the healthy controls as separate groups (Table 2). However, only protein C was significantly different between the two patient groups (74.5±5.1% for Pattern D vs. 61.6±3.3% for Pattern E+F; $p = 0.0124$).

The levels of D-dimers were significantly higher in the total patient group compared to controls (210 ng/mL [61–3,224 ng/mL] vs. 96 ng/mL [47–190 ng/mL]; median [range]; $p<0.001$) and as shown in Figure 1 higher levels were a common feature in the patients with advanced periportal fibrosis (229 ng/mL [60.7–3,224 ng/mL]). Using the cut-off value of 483 ng/mL for D-dimer as a measure of hyperfibrinolysis [14], we found that 11 of 38 patients (29%) with advanced periportal fibrosis (Pattern E+F) and a history of upper digestive bleeding exhibited D-dimer levels above this value.

PAI-1 levels were decreased in the patients compared to the control group (65 ng/mL [5–162] vs. 202 ng/mL [17–448 ng/mL]; median [range]; $p<0.001$), but there were no significant differences ($p>0.05$) in plasma levels of t-PA and TAFI (data not shown).

All controls and patients were negative for elimination of Schistosoma mansoni eggs in the stool, and also for hepatitis B and C virus markers.

Discussion

Periportal fibrosis is the main liver consequence of severe infection by *S. mansoni*. It plays a key role in the genesis of portal hypertension and in distorting hepatic parenchyma, which can cause hepatic dysfunction when the fibrosis is extensive. These effects may persist in some patients even after treatment and cure of the infection [7]. Moreover, bleeding episodes can allow progression to decompensated liver disease due to areas of hepatic necrosis caused by hypotension and loss of blood. In our study, we evaluated different patterns of advanced periportal fibrosis in patients with compensated hepatosplenic schistosomiasis, but without ascites, jaundice or hepatic encephalopathy. Pattern E [12] was the most prevalent, and 45% had a previous history of gastrointestinal bleeding. Consistent with our findings reported here, Correia et al [5] observed a high frequency (82%) of thrombocytopenia in patients with hepatosplenic schistosomiasis while other studies have demonstrated progressive deterioration of hepatic function in advanced stages of schistosomasis disease [9–11].

Although we report that central fibrosis (Pattern D) is already associated with liver damage, our findings also suggest that γ-GT levels are the best marker of hepatic fibrosis progression. This supports the use of γ-GT as one of three biological markers proposed by Camacho-Lobato and Borges [9] to evaluate the progression of liver dysfunction in schistosomiasis, while Köpke-Aguiar et al. [15] also found γ-GT to be a sensitive indicator along with the platelet count and INR to differentiate patients with or without portal hypertension. Moreover, elevated γ-GT along with ALP was also a feature in hepatosplenic patients with anicteric cholangiopathy, who have alterations in biliary ducts (ductopenia intermediary and small caliber branches) reflecting advanced fibrosis [16].

The liver play a major role in the control of hemostasis, and disturbed liver parenchymal cell function affects the hemostastic system. Such studies report a frequent thrombocytopenia associated with the splenomegaly and portal hypertension [17]. The thrombocytopenia in hepatosplenic schistosomiasis is compensated, at least in part, by increased levels of von Willebrand factor

Table 1. Liver function tests in hepatosplenic schistosomiasis patients with different patterns of periportal fibrosis.

Characteristics	Controls (C)	Hepatosplenic Schistosomiasis Patients			p-value			
		Overall	D fibrosis pattern	E+F fibrosis pattern	Overall vs. C	D vs. C	E+F vs. C	D vs E+F
Subjects (n)	29	55	17	38	-	-	-	
AST (U/L)	21.9±1.2	51.6±4.7	54.4±9.3	50.4±5.4	<0.0001	0.0003	0.0001	0.6276
ALT (U/L)	18.6±0.9	49.2±5.8	57.5±10.9	45.4±6.9	0.0003	0.0005	0.0027	0.2399
ALP (U/L)	65.2±3.4	170.7±23.5	162.3±21.4	174.4±32.8	0.0017	0.0287	0.0026	0.7716
γ-GT (U/L)	27.2±2.6	145.0±19.2	103.4±24.1	169.4±25.1	<0.0001	0.0313	<0.0001	0.0501
Albumin (g/dL)	4.30±0.07	3.98±0.08	4.03±0.12	3.96±0.11	0.0156	0.1189	0.0170	0.6834
Total bilirubin (g/dL)	0.65±0.05	1.23±0.12	1.18±0.16	1.26±0.16	0.0011	0.0239	0.0017	0.7345
Direct bilirubin (g/dL)	0.33±0.03	0.52±0.08	0.43±0.08	0.57±0.12	0.1050	0.5333	0.0667	0.3624
Indirect bilirubin (g/dL)	0.31±0.04	0.71±0.07	0.75±0.15	0.69±0.08	0.0002	0.0017	0.0008	0.6343

Values are expressed as mean±Standard Error (SE). Unpaired Student's *t* test or one-way ANOVA followed by Fisher's PLSD post test.
doi:10.1371/journal.pntd.0002314.t001

that enable platelets to adhere and aggregate at sites of vascular injury [5,18,19]. Köpke-Aguiar et al. [20] also reported that levels of thrombopoetin and reticulated platelets are normal in schistosomiasis patients with portal hypertension and that the bone marrow produces normal amounts of platelets. Thrombocytopenia in schistosomiasis patients may occur because of splenic retention due to poor portal blood drainage, or because platelets are trapped in the sinusoidal spaces of the fibrotic liver [20]. Our study confirms that thrombocytopenia is common in patients with hepatosplenic schistosomiasis, and that this tends to be higher in the advanced stages of periportal fibrosis.

A number of studies have demonstrated reduced vitamin K-dependent coagulation factors in patients with hepatosplenic schistosomiasis [9–11] and our findings agree with these reports. Several mechanisms may explain the substantial reductions in coagulation factors, including reduced hepatic synthesis and increased consumption. Impaired carboxylation of precursor molecules is also proposed for factors II, VII, IX and X [11], due to premature release of the protein from damaged hepatocytes, or because of vitamin K-dependent carboxylase deficiency and production of abnormal proteins [10,11]. Moreover, Tripodi et al. [21] recently highlighted the occurrence of concomitant decreases of both procoagulant and anticoagulant factors in chronic liver disease, mainly in cirrhotic patients. These features escaped attention for many years [11,21].

Levels of protein C and antithrombin were significantly lower in our patients compared to the healthy controls, presumably reflecting hepatic dysfunction caused by portal hypertension and advanced periportal fibrosis [10,20–22]. Although bleeding events in hepatosplenic schistosomiasis are associated with portal hypertension [20], the deficient production of coagulation factors does not seem to aggravate the situation due to a balance between the reductions in pro- and anti-coagulation proteins. Our results also show that in almost all cases the changes in blood coagulation

Table 2. Coagulation parameters from hepatosplenic schistosomiasis patients with different patterns of periportal fibrosis.

Characteristics	Controls (C)	Hepatosplenic Schistosomiasis Patients			p-value			
		Overall	D fibrosis pattern	E+F fibrosis pattern	Overall vs. C	D vs. C	E+F vs. C	D vs. E+F
Subjects (n)	29	55	17	38	-	-	-	-
Platelets Count ($\times 10^9$/L)	261.1±9.8	128.4±12.7	146.6±23.1	120.3±14.0	<0.0001	<0.0001	<0.0001	0.1112
INR	1.01±0.02	1.44±0.06	1.38±0.09	1.47±0.07	<0.0001	0.0028	<0.0001	0.4828
TT (Seconds)	11.9±0.2	13.8±0.2	13.56±0.4	13.85±0.3	<0.0001	0.0001	<0.0001	0.4770
PTT (Seconds)	31.1±0.6	37.9±1.5	36.4±1.9	38.6±2.0	0.0012	0.0510	0.0010	0.3969
Fibrinogen (mg/dL)	342.9±21.0	267.5±9.9	263.4±15.6	269.4±12.7	0.0002	0.0045	0.0007	0.9770
Factor II (%)	92.7±3.3	66.6±2.3	67.8±4.2	66.0±2.8	<0.0001	<0.0001	<0.0001	0.6377
Factor VII (%)	84.8±4.1	49.9±2.4	56.6±4.2	46.9±2.7	<0.0001	<0.0001	<0.0001	0.0839
Factor VIII (%)	120.2±6.5	90.5±4.2	96.3±8.2	87.9±4.8	0.0001	0.0164	<0.0001	0.3238
Factor IX (%)	100.1±3.3	59.9±2.5	65.3±4.5	57.5±2.9	<0.0001	<0.0001	<0.0001	0.1375
Factor X (%)	88.8±3.7	63.8±3.7	59.1±7.4	65.91±4.3	<0.0001	0.0001	0.0001	0.4842
Protein C (%)	100.1±2.2	65.6±2.9	74.5±5.1	61.6±3.3	<0.0001	<0.0001	<0.0001	0.0124
Antithrombin IIa (%)	112.0±3.4	92.8±3.5	96.0±8.8	91.3±3.3	<0.0001	0.0319	0.0007	0.4560

Values are expressed as mean±Standard Error (SE). Unpaired Student's *t* test or one-way ANOVA followed by Fisher's PLSD post test.
doi:10.1371/journal.pntd.0002314.t002

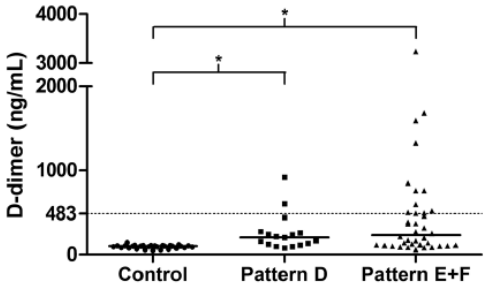

Figure 1. Plasma D-dimer levels in healthy control subjects (Control) and hepatosplenic schistosomiasis patients with either central fibrosis (Pattern D) or advanced periportal cirrhosis (Pattern E+F). The solid horizontal lines show D-dimer median values (96, 199 and 229 ng/mL), while the dashed line indicates the cut-off value for hyperfibrinolysis [14]. *p<0.001; Kruskal-Wallis test.
doi:10.1371/journal.pntd.0002314.g001

proteins were greater in patients with advanced periportal fibrosis (Pattern E+F) than those with central fibrosis (Pattern D), although statistical significance for the difference was only reached for protein C. Nevertheless, our data suggest that platelet counts, Factor VII and protein C are good predictors of advanced fibrosis.

Fibrinolysis was studied in patients with decompensated hepatosplenic schistosomiasis by El-Bassiouni et al. [23] who reported high concentrations of t-PA and low levels of PAI-1. Although PAI-1 was also decreased in our patients, the level of t-PA (and TAFI) did not differ between our patients and controls. In addition, we found increased levels of D-dimer, most notably in those patients with advanced periportal fibrosis (Pattern E+F). Plasma D-dimer concentration reflects the degree of thrombin turnover and consequently increased levels are a good marker of recent coagulation and fibrinolysis. Indeed, Primignani et al. [14] suggested D-dimer as a predictor of death in patients with liver cirrhosis. Using the cut-off value proposed by Primignani et al. [14], one-third of our patients with Pattern E+F were considered to have hyperfibrinolysis, which may increase their risk of a bleeding event. Therefore, we recommend for patients eligible for surgical procedures that D-dimer be measured during the pre-surgical evaluation, in addition to PT and platelet count, to better assess the risk of bleeding.

One limitation of our study is that it was conducted at a single hospital, the Hospital das Clinicas, UFPE. This is the reference hospital for schistosomiasis in Pernambuco State and receives the most severe cases of schistosomiasis, usually patients with a history of one or more episodes of gastrointestinal bleeding and hence a high proportion with abnormal liver function tests. Thus, the findings from our study may not extrapolate to all patients from endemic areas who present with the hepatosplenic form of the disease.

In conclusion, our study verified that coagulation abnormalities in hepatosplenic schistosomiasis are due to liver fibrosis and portal hypertension, and additionally demonstrated that these abnormalities increase in advanced periportal fibrosis and that reduced levels of protein C may be a good marker of hepatic fibrosis progression.

Supporting Information

Checklist S1 STROBE checklist.
(DOC)

Author Contributions

Conceived and designed the experiments: LACL VLdML ALCD EPL. Performed the experiments: LACL AAPF SMLM. Analyzed the data: LACL CSMdF BSdS RdCdSF EPL ALCD VLdML. Contributed reagents/materials/analysis tools: VLdML. Wrote the paper: LACL CSMdF BSdS EPL ALCD VLdML JSO.

References

1. Yuesheng Li, Chen D, Ross AG, Burke ML, Xiling Yu, et al. (2011) Severe hepatosplenic schistosomiasis: clinicopathologic study of 102 cases undergoing splenectomy. Hum Pathol 42: 111–149.
2. Ross AGP, Bartley PB, Sleigh AC, Olds GR, Li Y, et al. (2002) Schistosomiasis. N Engl J Med 346: 1212–1220.
3. Olliaro PL, Vaillant MT, Belizario VJ, Lwambo NJS., Ouldabdallahi M, et al. (2011) A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs. 60 mg/kg for treating intestinal schistosomiasis in the Philippines, Mauritania, Tanzania and Brazil. PLoS Negl Trop Dis 2: e1165.
4. Coutinho EM, Abath FGC, Barbosa CS, Domingues ALC, Melo MCV, et al. (1997) Factors involved in schistosoma infection in rural areas of Northeast Brazil. Mem Inst Oswaldo Cruz 92: 707–715.
5. Correia MCB, Domingues ALC, Lacerda HR, Santos EM, Machado CGF, et al. (2009) Platelet function and the von Willebrand factor antigen in the hepatosplenic form of schistosomiasis mansoni. Roy Soc Trop Med Hyg 103: 1053–1058.
6. Andrade ZA (2009) Schistosomiasis and liver fibrosis. Parasite Immunol 31: 656–663.
7. Ferraz AAB, Albuquerque PC, Lopes EPA, Araujo Jr JGC, Carvalho AHF, et al. (2003) The influence of periportal (Pipestem) fibrosis on long term results of surgical treatment for schistosomotic portal hypertension. Arq Gastroenterol 101: 183–186.
8. Gryseels B, Polman K, Clerinx K, Kestens L (2006) Human schistosomiasis. Lancet 368: 1106–18.
9. Camacho-Lobato L, Borges DR (1998) Early liver dysfuction in schistosomiasis. J Hepatol 29: 233–240.
10. Tanabe M (2003) Haemostatic abnormalities in hepatosplenic schistomomiasis mansoni. Parasitol Int 52: 351–359.
11. Omran SA, Amin HM, El-Bassiouni NE, Essawy FM, Toiema SM (1994) Vitamin K dependent coagulation proteins in endemic hepatosplenomegaly in Egypt. J Clin Pathol 47: 502–504.
12. Niamey Working Group (2000) Ultrasound in schistosomiasis: a practical guide to the standardized use of ultrasonography for the assessment of schistosomiasis-related morbidity. Geneva: World Health Organization. TDR/STR/SCH/00.1.
13. Richter J, Domingues ALC, Barata CH, Prata AR, Lambertucci JR (2001) Report of the Second Satellite Symposium on Ultrasound in Schistosomiasis. Mem Inst Oswaldo Cruz 96; 151–156.
14. Primignani M, Dell'Era A, Bucciarelli P, Bottasso B, Bajetta MT, et al (2008) High-D-dimer levels predict poor outcome in esophageal variceal bleending. Dig Liver Dis 40: 878–881.
15. Kopke-Aguiar LA, Martins JRM, Passeotti CC, Toledo CF, Nader HD, et al. (2002) Serum hyaronic acid as a comprehensive marker to assess severity of liver disease in schistosomiasis. Acta Trop 84: 117–126.
16. Brandt PE, Kopke-Aguiar LA, Shigueoka DC, Sales D, D'Ippolito G, et al. (2008) Anicteric cholangiopathy in schistosomiasis patients. Acta Trop 108: 218–221.
17. McCormick PA, Murphy KM (2000) Splenomegaly, hypersplenism and coagulation abnormalities in liver disease. Baillière Clin Gastro 14: 1009–1031.
18. Petroianu A, Oliveira AE, Alberti LR (2004) "Hiperesplenismo" em hipertensão porta por esquistossomose mansônica. Rev Bras Hematol Hemoter 26: 195–201.
19. Nakamura M, Shibazaki M, Nitta Y, Endo Y (1998) Translocations of platelets into Disse spaces and their entry into hepatocytes in response to lipossaccharides, interleukin-1 and tumour necrosis factor: The role of Kupffer cells. J Hepatol 28: 991–999.
20. Köpke-Aguiar LA, de Leon CP, Shigueoka DC, Lourenço DM, Kouyomdjian M, et al. (2009) Reticulated platelets and thrombopoetin in schistosomiasis patients. Int J Lab Hematol 31: 69–73.
21. Tripodi A (2010) The coagulopathy of chronic liver disease: Is there a causal relationship with bleeding: No. Eur J Int Med 21: 63–69.
22. Borges DR, Manoukian N, Toledo CF (1987) Protein C deficient in the compensated form of hepatosplenic schitosomiasis. Braz J Med Biol Res 20: 557–560.
23. El-Bassiouni NE, El-Bassiouni AE, Khayat HE, Akl MM, Omran SA (1996) Hyperfibrinolysis in hepatosplenic schistomiasis. J Clin Pathol 49: 990–993.

CAPÍTULO II

- Journal List
- Rev Bras Hematol Hemoter
- v.35(5); 2013
- PMC3832313

Rev Bras Hematol Hemoter. 2013; 35(5): 332–336.

doi: 10.5581/1516-8484.20130098

PMCID: PMC3832313

Relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis

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Background: *Schistosomiasis is a tropical disease. Patients who develop hepatosplenic schistosomiasis have clinical findings including periportal fibrosis, portal hypertension, cytopenia, splenomegaly and gastrointestinal hemorrhage.*

Objective: *The aim of this study was to analyze the hemostatic and hematologic findings of patients with schistosomiasis and correlate these to the size of the spleen.*

Methods: *Fifty-five adults with hepatosplenic schistosomiasis and 30 healthy subjects were selected through a history of contact with contaminated water, physical examination and ultrasound characteristics such as periportal fibrosis and splenomegaly in the Gastroenterology Service of the Universidade Federal de Pernambuco. Blood samples were collected to determine liver function, blood counts, prothrombin (international normalized ratio), partial thromboplastin time and fibrinogen and D-Dimer levels using the Pentra 120 hematological analyzer (HORIBA/ABX), Density Plus (test photo-optical Trinity Biotech, Ireland) and COBAS analyzer 6000 (Roche). Furthermore, the longitudinal size of the spleen was measured by ultrasound (Acuson X analyzer 150, Siemens). The Student t-test, the Fisher test and Pearson's correlation were used to analyze the results with statistical significance being set for a p-value < 0.05.*

Results: *The mean age was higher for the Study Group than for the Control Group (54 ± 13.9 vs. 38 ± 12.7 years). The average longitudinal diameter of the spleen was 16.9 cm (Range: 12.3-26.3 cm). Anemia is a common finding in patients with schistosomiasis (36.3%). The mean platelet and leukocyte counts of patients were lower than for the Control Group (p-value < 0.001). Moreover, the international normalized ratio (1.42 vs. 1.04), partial thromboplastin time (37.9 vs. 30.5 seconds) and D-Dimer concentration (393 vs. 86.5 ng/mL) were higher for the Study Group compared to the Control Group.*

Conclusion: *This study suggests that hematological and hemostatic abnormalities are associated with splenomegaly, hypersplenism and portal hypertension.*

Keywords: *Schistosomiasis mansoni; Schistosomiasis; Splenomegaly; Hypersplenism; Thrombocytopenia*

Introduction

Schistosomiasis mansoni is a chronic parasitic disease and the most prevalent tropical liver disease in the northeastern region of Brazil. There are 200 million people affected by *Schistosoma mansoni* worldwide with 600 million people being exposed. Around 5-7% of the patients infected by *S. mansoni* progress to the most severe form, hepatosplenic schistosomiasis (HS). Many of these patients exhibit high morbidity associated with periportal fibrosis, portal hypertension, splenomegaly, upper digestive tract bleeding and cytopenia[1-5].

Hypersplenism is a consequence of massive splenomegaly and is a common finding in chronic liver diseases. In schistosomiasis, this results from hyperplasia of the reticuloendothelial system and consequently venous congestion caused by portal hypertension. Studies have reported a correlation between the increase of spleen size and drops in blood cell counts, mainly the platelet count. These findings depend on the severity of portal hypertension as some studies have shown that thrombocytopenia is more common in HS patients, especially after episodes of digestive tract bleeding[6-8].

Some studies have reported that compensated HS patients exhibit normal hepatic function even with some abnormalities in blood clotting. Other reports have called attention to hemostatic abnormalities in decompensated HS patients who develop ascites and upper digestive tract bleeding including increases in the prothrombin time/international normalization ratio (PT/INR), partial thromboplastin time (PTT), thrombin time (TT) and abnormalities in K-dependent factors. The liver plays an important role in the control of blood coagulation and disorders of parenchymal liver cell function affect the hemostatic system. Liver disorders may be associated with the reduction of coagulation protein synthesis, in particular in cirrhosis patients. These findings are still unclear and represent a contradiction for medical research, especially in studies involving liver diseases such as cirrhosis and schistosomiasis and other liver disorders that lead to progressive hepatic damage[9-11].

The purpose of this study was to evaluate the hematological and hemostatic abnormalities in patients with severe forms of schistosomiasis, and possible associations with splenomegaly and portal hypertension.

Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 9/26/2012
Accepted: 3/14/2013

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DOI: 10.5581/1516-8484.20130098

Methods

Diagnosis

A prospective study was undertaken involving 55 compensated HS patients previously treated using praziquantel (50 mg/kg) and 30 healthy subjects in the Outpatient Gastrointestinal Service of *Hospital das Clínicas* of the *Universidade Federal de Pernambuco* (UFPE), Recife, Brazil from 2010 to 2012. Patients and controls had comparable socioeconomic conditions including similar salaries, educations and lifestyles. The diagnosis and consecutive selection of HS patients was based on clinical history, physical examination and an abdominal ultrasonography that showed periportal fibrosis and splenomegaly. The abdominal ultrasound was performed using an ultrasound analyzer (Acuson X 150, 3.5 MHz, Siemens). For this, we used the Cairo and Niamey protocols to measure the longitudinal diameter of the spleen and classify the pattern of fibrosis as central fibrosis (Pattern D), advanced fibrosis (Pattern E) or very advanced fibrosis (Pattern F)[12-14].

Upper digestive tract endoscopy was used either to confirm or exclude the presence of esophageal varices and viral marker and liver function tests were performed to exclude viral hepatitis B and C. Patients suffering from alcoholism (> 60 g/day for men and > 40 g/day for women)[15], systemic diseases such as diabetes mellitus, collagenosis, blood diseases (lymphoproliferative disease and lymphomas), those that were taking hepatotoxic, antiaggregant or anticoagulant drugs and every patient with a history of splenectomy or blood transfusions (within the previous 3 months) were carefully excluded. Stool samples were examined to detect intestinal parasites by the Lutz-Hoffman followed by Kato-Katz method. Routine liver tests included analysis of aspartate transaminase (AST) alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (γGT) and albumin. The Control Group had the same exclusion criteria as described above and were checked for possible contact with water contaminated by the cercariae of *S. mansoni* and positive parasitological tests. The compensated hepatosplenic form corresponds to the severe form of disease and includes patients with hepatosplenomegaly and portal hypertension, but without ascites, jaundice or encephalopathy[9].

Sample collection

About 15 mL of venous blood was drawn under aseptic conditions without stasis and placed in vacuum tubes (Vacutainer; Becton Dickinson, UK). The first blood sample was placed in a polypropylene tube containing 0.106 M trisodium citrate at a blood-anticoagulant ratio of 9:1. These tubes were used for the coagulation tests (PT, PTT, fibrinogen and D-dimer). The blood samples were centrifuged at 2000 g for 15 minutes at 4°C. Subsequently, the platelet free plasma was quickly distributed in 0.5 mL aliquots in plastic-capped tubes and stored at -80ºC for six months until processing. A second sample, placed in a tube containing ethylenediaminetetraacetic acid (EDTA), was used for the complete blood count (CBC). A blood smear was prepared and stained for conventional microscopic analysis. The CBC was carried out by electrical impedance or light dispersion using a Pentra 120 analyzer (ABX, São Paulo, SP, Brazil). A third sample was collected for routine liver tests (AST, ALT, γGT, ALP, albumin) using biochemistry tubes (Becton Dickinson, UK) and the assay was performed using the COBAS 6000 analyzer (Roche). The aliquots of plasma serum samples were also stored for the other tests. The hemostatic tests (PT/INR, PTT, fibrinogen and D-dimer) were carried out in an automated photo-optical coagulometer, (Trinity Biotech, Acton, Ireland) following the manufacturer's instructions. The study was conducted according to the norms of the Declaration of Helsinki. The protocols for the collection and use of human samples were submitted and approved by the Research Ethics Committee of the Health Sciences Center of the UFPE (Nº 028/11). All subjects received an explanation about the study and signed informed consent forms.

Statistical analysis

Statistical analyzes were performed using the unpaired Student t-test and Fisher's test for contingency analysis. These analyzes were performed using the Statistical Package for Social Sciences software (SPSS 17.0, Chicago, IL, USA). Continuous variables were expressed as means ± standard deviation (SD), median and range. Furthermore, the Pearson correlation test was employed to examine the relationship between the longitudinal diameter of the spleen and platelet counts. P-values of less than 0.05 were considered statistically significant.

Results

The results are shown in Tables 1 & 2 and in Figures 1 to 4. The patients had a mean age of 54 ± 13.9 years and the 30 control subjects had a mean age of 38.3 ± 13.7 years (p-value < 0.001) but no difference was found in respect to the gender. The Niamey

Table 1 - Liver function tests in hepatosplenic schistosomiasis patients and the Control Group

Variable	Control Group	Hepatosplenic Group	p-value
Participants	30	55	
AST (units/L) median (range)	20 (15-29)	39 (15-201)	< 0.0001
ALT (IU/L) median (range)	17.5 (9-27)	36 (12-257)	< 0.0001
ALP (IU/L) median (range)	67.0 (28-105)	132 (58-1321)	< 0.0001
γGT (IU/L) median (range)	24.5 (9-75)	93 (16-756)	< 0.0001
Albumin (g/L) (mean ± SD)	43 ± 5	38 ± 6	< 0.0001

AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; γGT: gamma-glutamyl transferase

Table 2 - Distribution of the hepatosplenic schistosomiasis patients according to type of cytopenia

Cytopenias	n	%
No cytopenia	12	21.8
Leukopenia in isolation	4	7.27
Thrombocytopenia in isolation	12	21.8
Bicytopenia	17	30.9
Pancytopenia	10	18.1
Total	55	100

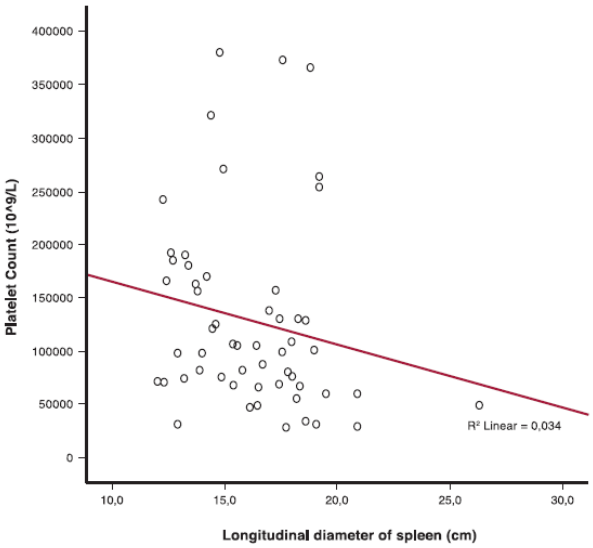

Figure 1 – Relationship between longitudinal diameter of the spleen and platelet counts in hepatosplenic patients

A direct positive correlation was observed between splenomegaly and platelet counts in schistosomiasis patients (r2 = 0.034; p-value = 0.02)

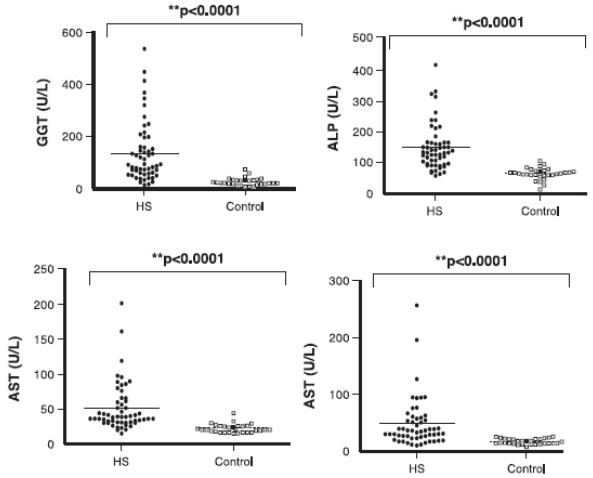

Figure 3 – Routine liver tests in the Control and Schistosomiasis Groups

Data are expressed as means. Controls vs. hepatosplenic schistosomiasis (HS) patients
AST: Aspartate transaminase; ALT: Alanine transaminase; γGT: gamma-glutamyl transferase

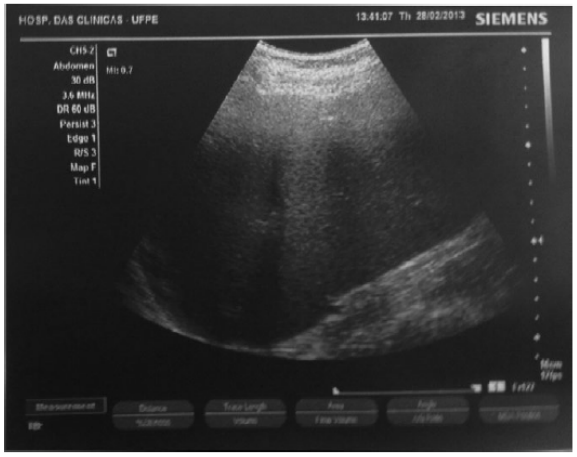

Figure 2 – Image showing a massive splenomegaly (18.7 cm) in a hepatosplenic schistosomiasis patient

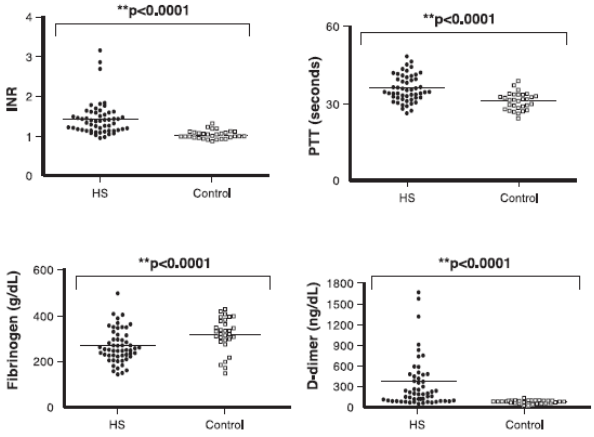

Figure 4 – Coagulation and fibrinolytic parameters in Control and Schistosomiasis Groups

Data are expressed as mean
Controls vs. hepatosplenic schistosomiasis (HS) patients
IRN: international normalized ratio; PTT: partial thromboplastin time;
The D-dimer concentration was used for measure the fibrinolytic status and risk of bleeding

classification of fibrosis showed predominance of advanced (pattern E - 30 patients; 54.5%), followed by pattern D (17 patients; 30.9%) with only eight patients (14.5%) having very advanced fibrosis (pattern F). The routine liver tests showed significant differences in relation to parameters such as albumin, AST, ALT, ALP and γGT between the groups (p-value < 0.001; Figure 1).

Upper gastrointestinal bleeding was found in 34 (61.8%) patients. The mean longitudinal diameter of the spleen of the 55 patients was 16.9 cm (Range: 12.3-26.3 cm) and the median platelet count was 101.0 x 10^9/L. A total of 38 (69.1%) patients had thrombocytopenia (platelet counts below 150 x 10^9/L). Furthermore, 14.5% of patients had platelet counts below 50 x 10^9/L (Table 1). There was an inverse correlation between the longitudinal diameter of the spleen and the platelet count (Figure 2). It was also found that 36.3% of patients had anemia and 47.3% presented leukopenia (cytopenias were different in each

patient). Table 2 shows the distribution of HS patients according to the type of cytopenia. Figure 3 demonstrates the massive splenomegaly of one patient with schistosomiasis.

Figure 4 demonstrates that HS patients presented with increased INR, PTT, and fibrinogen and D-dimer concentrations. There were significant differences in respect to these parameters on comparing the Control Group with patients (p-value < 0.001).

Discussion

Portal hypertension is one of the most important consequences of *S. mansoni* infections. Due to the fibrotic process and venous congestion, HS patients develop hemodynamic changes associated with splenomegaly and high morbidity rates[16]. In a recently study in the same region, Dias et al.[17] showed that 61% of HS patients had advanced or very advanced periportal fibrosis. In the current study, all HS patients presented with a severe form of HS and portal hypertension, 38 (69%) had advanced and very advanced liver fibrosis (pattern E+F) and 34 (61.8%) patients had gastrointestinal bleeding. This proves that these patients have advanced disease and that changes in liver function tests may be related to the high frequency of advanced fibrosis.

Camacho-Lobato et al. and Tanabe reported that a large number of HS patients present conserved liver function even with periportal fibrosis[9,11]. However, this study demonstrates elevated liver function tests and remarkable changes in coagulation tests. Some studies have reported varied frequencies of thrombocytopenia (30 to 75%) depending on the stage of portal hypertension[8-16]. Lower values of platelet counts are seen especially in patients with HS after gastrointestinal bleeding. This was also seen in the current work with the possible cause being the increase of spleen and hypersplenism leading to frequent thrombocytopenia, leukopenia and anemia.

Thrombocytopenia is a common feature in chronic liver disease. Correia et al.[8] showed that thrombocytopenia can be compensated by increased levels of von Willebrand factor in HS patients. Other studies have shown that nearly 50% of HS patients exhibit thrombocytopenia, 16% bicytopenia and 7% anemia or leukopenia[18]. Anemia in patients with liver disease is often multifactorial and may be associated with iron and folic acid deficiency or even an inflammatory process. Red blood cell survival is often shortened in chronic liver disease with the increased spleen being a major site of red blood cell destruction[17]. In this study, high frequencies of thrombocytopenia (74.1%), leukopenia (47.3%), anemia (36.3%), bicytopenia (30.9%) and pancytopenia (18.2%) were found. The high frequencies of cytopenias found may be associated with the increased spleen size and the consequent hypersplenism leading to an increase in scavenging and retention of blood cells in the spleen, mainly platelets and white blood cells. Furthermore, anemia may be accentuated because of the low socioeconomic situation of the patients and due to blood loss from upper digestive tract bleeding.

Berzigotti et al.[19] used the diameter of the spleen combined with platelet count as a noninvasive marker of portal hypertension; these tools can also be used to predict esophageal varices and upper gastrointestinal bleeding episodes, especially in asymptomatic patients with cirrhosis. In this study, an inverse relationship was found between platelet count and longitudinal diameter of the spleen. Concomitant enlargement of the spleen from 15 to 20 cm together with a platelet count below 100 x 10^9/L is an excellent predictor of portal hypertension, esophageal varices and upper digestive tract bleeding, even in asymptomatic patients with schistosomiasis. These two parameters combined with an increased INR could be a useful noninvasive tool to predict a different behavior of portal hypertension in patients with HS. Nevertheless, further studies are needed to identify other noninvasive markers and thus validate a score to classify the different stages of schistosomiasis and other liver diseases.

A recent study showed that the levels of thrombopoietin and reticulated platelets were normal in schistosomiasis patients with portal hypertension and that the bone marrow produces platelets normally[20]. The cytopenias in schistosomiasis patients may occur because the cells are retained in the spleen due to difficulty in draining portal blood. Additionally, platelets may be retained in the sinusoidal spaces of the fibrotic liver. Some studies have shown that the degree of liver fibrosis is associated with the degree of esophageal varices and that the risk of bleeding is associated with the degree of liver fibrosis[21]. Studies by Köpke-Aguiar et al.[20] report that there are no changes in the production of platelets in the bone marrow in schistosomiasis patients.

Conclusion

The present study suggests that the hematological abnormalities seen in HS patients are associated with splenomegaly, hypersplenism and portal hypertension. However, further studies are needed to verify whether the platelet count might be a non-invasive tool to assess portal hypertension.

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Ciência e Tecnologia de Pernambuco, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

References

1. Ross AG, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, et al. Schistosomiasis. N Engl J Med. 2002;346(16):1212-20. Comment in: N Engl J Med. 2002;347(10):766-8; author reply 766-8.
2. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet. 2006; 368(9541):1106-18.
3. Coutinho EM, Abath FG, Barbosa CS, Domingues AL, Melo MC, Montenegro SM, et al. Factors involved in Schistosoma mansoni infection in rural areas of northeast Brazil. Mem Inst Oswaldo Cruz. 1997;92(5):707-15.
4. Silva CA, Oliveira KF, Carvalho VC, Domingues AL, Brandt CT, Lima VL. Surgical treatment effect on the liver lecitin: cholesterol acyltransferase (LCAT) in schistosomiasis mansoni. Acta Cir Bras. 2001;17(Supl. 1):28-30.
5. Ferraz AA, Albuquerque PC, Lopes EP, Araújo Jr JG, Carvalho AH, Ferraz EM. The influence of periportal (pipestem) fibrosis on long term results of surgical treatment for schistosomotic portal hypertension. Arq Gastroenterol. 2003;40(1):4-10.

CAPÍTULO III

Evidence of changes in liver function as measured by blood coagulation factors in hepatosplenic schistosomiasis patients and subjected to spleen surgery

Impact Factor: 3.87

Evidence of changes in liver function as measured by blood coagulation factors in hepatosplenic schistosomiasis patients and subjected to spleen surgery

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Abstract

Background: Schistosomiasis is a chronic parasitic liver disease and some patients infected by *S. mansoni* progress to the most severe form, hepatosplenic. This form presents high morbidity associated with portal hypertension and splenomegaly, which leads to episodes of gastrointestinal bleeding. Moreover, the patients with the hepatosplenic form have abnormalities in blood coagulation and fibrinolysis proteins even with liver function preserved. Splenectomy is a good alternative to reduce portal hypertension, after digestive bleeding. The purpose of this study was to compare the liver function tests and blood coagulation factors between hepatosplenenic form and splenectomized patients.

Patients and Methods: 55 patients with hepatosplenic form and 45 splenectomized patients were evaluated by clinical and ultrasound examination. Blood samples were obtained for liver function tests, platelets count and prothrombin time. The blood coagulation factors (II, VII, VIII, IX and X), protein C and antithrombin IIa, plasminogen activator inhibitor 1 were measured by photometry, chromogenic and enzyme-linked immunosorbent assay. Hyperfibrinolysis was defined on the basis of plasminogen activator inhibitor 1 levels.

Results: Both groups of schistosomiasis patients exhibited similar age, gender and pattern of periportal fibrosis, but portal vein diameter was increased in hepatosplenic patients. Furthermore, the alkaline phosphatase were elevated in hepatosplenic patients when compared to splenectomized patients, while for coagulation factors there was an increase of prothrombin time, thromboplastin time and lower levels of factor II, VII, VIII, IX, X and protein C in hepatosplenic patients compared to the splenectomized. The concentration of plasminogen activator inhibitor 1 was higher in splenectomized than in hepatosplenic schistosomiasis patients.

Conclusion: This study suggests that there are some abnormalities in liver function tests and blood coagulation proteins levels in hepatosplenic schistomosiasis patients and that these alterations are not observed in splenectomized patients. Probably these dysfunctions of liver function tests and blood coagulation factors may be influenced by portal hypertension in hepatosplenic patients.

Keywords: portal hypertension – *Schistosoma mansoni* – splenectomy - blood coagulation - fibrinolysis

Schistosomiasis causes one of the most prevalent liver diseases, affecting more than 200 million people in over 74 different countries and is a major public health problem in the Northeast region of Brazil (1-3). Nearly 3 to 7% of patients infected by *S. mansoni* progress to the most severe

form, hepatosplenic schistosomiasis (HS), which is characterized by periportal fibrosis (PPF), obstrution by eggs of the intrahepatic veins, presinusoidal portal hypertension, splenomegaly and hemodynamic abnormalities, resulting in upper digestive bleeding (3-4).

Upon blocking the terminal branches of the portal vein, the deposition of numerous eggs of *S. mansoni* provoked the granulomas reactions with subsequent fibrosis, intrahepatic portal vein obstrution and increased resistance to the flow of blood to the sinusoids (5). Splenomegaly results as much from the congestion caused by the obstruction of the eggs and fibrosis as by the hyperplasia of the cells of the reticulo-endothelial system induced by immunological stimulation caused by the antigens released by the worms and eggs (6-7). In addition, it has been reported that splenomegaly leads to thrombocytopenia in more than 60% of patients with HS schistosomiasis, associated with hypersplenism, especially in the advanced stages of the disease (8).

Both the increased resistance to portal outflow and the hyperflux in the spleno-portal territory as a result of the great splenomegaly trigger presinusoidal portal hypertension (9-11). In schistosomiasis mansoni, PPF forms around the portal branches, maintaining the architecture of the hepatic parenchyma conserved and the capacity for synthesis of hepatocytes normal (5, 12). However, the PPF may induces a lightly elevations of liver enzimes as ALP and γGT.

The splenectomy with ligation of the left gastric vein has become a good therapeutic option to reduce portal hypertension after episodes of gastrointestinal bleeding (7, 11).

It has been reported that some patients with advanced stage of schistosomiasis have abnormalities in hemostasis and mechanisms of fibrinolysis such as prologantion of prothrombin time (PT), thromboplastin time (PPT), thrombin time (TT) as well as thrombocytopenia, hypofibrinogenemia and decreases of vitamin-K-dependent factors, which must be related to the low degree of disseminated intravascular coagulation (12-14).

Moreover, some studies have shown that patients with HS schistosomiasis feature hyperfibrinolysis with increased levels of D-dimer (DD) and tissue plasminogen activator (t-PA)

and reduction of plasminogen activator inhibitor-1 (PAI-1), with a consequent tendency of bleeding (15). On the other hand, after splenectomy with ligation of the left gastric vein and esophagogastric disconnection 13 to 53% of patients with HS schistosomiasis developing portal vein thrombosis (11, 16).

The purpose of this study was to compare serum levels of liver enzymes and hemostatic profile of patients with HS schistosomiasis and those that had undergone splenectomy.

Patients and methods

Patients

Between April, 2011 and December, 2012, one hundred schistosomiasis patients (55 patients with HS schistosomiasis and 45 patients that had been splenectomized) were consecutively selected and attended at the outpatient clinic at the Gastroenterology Division, "Hospital das Clínicas" of the Federal University of Pernambuco, Recife, Brazil. All patients had been previously treated with praziquantel (50 mg/kg).

The diagnosis of schistosomiasis was based on clinical history, earlier contact with water in the endemic zone, history of positive parasitology for *S. mansoni*, specific treatment and ultrasound examination, revealing PPF. Abdominal ultrasound was performed by a single researcher (ALCD) through the Acuson X 150 device, with convex transducer of 3.5 mHz (Siemens) for diagnosing mansoni schistosomiasis, classifity the diferentes patterns of PPF, and to avoid confusion with other liver diseases such as steatosis and cirrhosis. The Niamey classification was used to establish the PPF patterns: pattern D (central fibrosis), pattern E (advanced fibrosis) and pattern F (very advanced fibrosis) (17-18).

Patients were excluded if they reported alcohol abuse (>60 g/day of ethanol for men and 40 g/day for women), pregnancy, diabetes mellitus, hepatitis B and C, fat liver diseases, cirrhosis, collagenosis, chronic lymphoproliferative diseases, and use of hepatotoxic, antiplatelet or anticoagulant drugs were also excluded. An occurrence of blood transfusion during 90 days preceding data collection also constituted an exclusion factor. All patients were tested for markers of hepatitis B virus (HBsAg and anti-HBc), hepatitis C (anti-HCV) and HIV (anti-HIV).

All patients received explanation about the study and signed a free and informed consent form. The study was approved by the Ethics Committee for Research on Humans at the Federal University of Pernambuco, Brazil (Number 028/11), in accordance with the Helsinki Declaration of 1975.

Collection and processing of samples

Venous blood samples were collected aseptically with minimal stasis using vacuum tubes (Vacutainer, Becton Dickinson, USA). Blood was collected into a tube containing 0.109 M trisodium citrate at a ratio of 9:1 for anticoagulant analysis and another containing 0.369M 3k ethylenediaminetetraacetic acid (EDTA-K3), which was used for the quantification of platelets. The last tube (serum tube) was used to evaluate of liver function tests, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl-transferase (γGT), bilirrubins and albumin. The blood samples were centrifuged, except for the second tube, for 10 minutes at 2000 *g* and the plasma and serum were divided into aliquots of 0.5 mL in plastic tubes and stored at -80°C until assayed.

Biochemical and coagulation tests

The serum concentration of each enzyme was divided by the value of normality according to sex (AST 31 IU/L for women and 35 IU/L for men; ALT 31 IU/L for women and 41 IU/L for man; γGT 38 IU/L for women and 55 IU/L for men and ALP 128 IU/L for women and 141 IU/L for men), with the values expressed by the ratio of the value found divided by the upper limit of normal (IU/L/ULN). The bilirubins and albumin were expressed in g/L. All liver function tests were measured spectrophotometrically through automated methods (6000 analyzer series Cobas®, Roche, USA). The HBsAg, anti-HBc, anti-HCV and anti-HIV markers were detected by immuno-enzymatic assay (ARCHITECT c8000, Abbott, USA).

The platelet counts were measured by electrical impedance (Pentra DF 120, HORIBA ABX SAS Diagnostics, Brazil). Coagulation tests were performed by the chromogenic method using a Destiny Plus automatic analyzer (Trinity Biotech, Ireland). Initially, PT, PTT, TT and the determination of fibrinogen were performed.

The quantification of coagulation factors (II, VII, VIII, IX, X), protein C, antithrombin IIa and DD were also measured using the Destiny Plus automatic analyzer (Trinity Biotech, Ireland). Additionally, assays to quantify the tests of fibrinolysis, t-PA, PAI-1 and TAFI (thrombin activatable fibrinolysis inhibitor) were determined by enzyme-linked immunosorbent assay - ELISA (Asserachrom Diagnostica, Stago, France).

Statistical analysis

Continuous variables are given as mean and Standard Error of the mean or median and range. Unpaired Student's *t* test was used to compare differences between normally distributed variables in hepatosplenic and splenectomized schistosomiasis patients, while Manny-Whitney test

was done for comparison test non-normally distribuited variables. The Pearson chi-square test was also used to compare the different patterns of periportal fibrosis. All statistical analyses were performed using Statview SAS Inc. (1998, NC, USA) and the graphs drawn was done using GraphPad Prism 6.0 (GraphPad Software Inc, CA, USA). P-values < 0.05 were considered statistically significant.

Results

In this study, the groups of 100 patients with schistosomiasis did not show significant differences in relation to age and sex. The PPF pattern was also similar in both groups. However the diameter of the portal vein in patients with 55 HS schistosomiasis patients was greater than in patients of the 45 splenectomized patients (Table 1). The average time of splenectomy for patients was 11.5±8.6 years, ranging from 2 to 33 years, and a median of 9 years.

Serum levels of AST, ALT, γGT and albumin were not different among the patients of the two groups. However, HS patients showed increased levels of ALP and total, direct, and indirect bilirubins (Table 2).

Patients of the splenectomized patients had platelet numbers greater than HS patients. Also it was found that patients in the splenectomized patients exhibited less prolonged PT and PTT than those of the HS group. Furthermore, levels of coagulation factors VII, VIII, IX, X, and protein C were observed to be higher in the splenectomized patients than in the HS group. Significant differences were not found in thrombin time and levels of ATIIa and fibrinogen between the two groups (Table 3). Figure 1 demonstrates that plasma levels of PAI-1 were greater in splenectomized patients.

Discussion

Portal hypertension is a major consequence of *S. mansoni* infection in terms of morbidity and mortality, due to the possibility of bleeding from esophageal or gastric varices (19). Splenectomy has been used as a therapeutic tool to improve hemodynamic conditions, treating and preventing new episodes of gastrointestinal bleeding, and results in a great reduction of pre-sinusoidal portal hypertension (20).

This study compared serum levels of liver enzymes and coagulation tests in HS schistosomiasis patients and who have undergone splenectomy. It is worth noting that the HS and splenectomized patients were comparable in terms of age, sex and pattern of PPF, but we have found differences in the liver function test between the groups.

Some mechanisms have been proposed to explain the elevation of ALP and γGT in HS patients, including the compression of small intrahepatic bile ducts by schistosomal granulomas (21). Studies by Amaral et al. (2002), however, did not detect changes in intra or extra-hepatic biliary tracts using ultrasound examination (22). Recent study from our group has demonstrated that levels of yGT increased according to patterns of PPF progression, suggesting that this enzyme may be a useful marker for stratifying the different patterns of periportal fibrosis (3). The intensity of the PPF depends on the immunogenetic response of the host associated with the degree of infection. The immunogenetic response and the severity of PPF encourage a greater increase in splenic volume and, consequently, greater hyperflux in the spleno-portal region, raising portal hypertension (9-11).

In fact, Alves et al, 2003 demonstrated that the greater the portal blood flow the higher the levels of ALP and γGT, associating these findings to possible anatomical changes in the biliary tree, arising from fibrosis in the portal region (23). Additionally, Toledo et al. (2009) observed higher

levels of ALP serum among schistosomiasis patients with portal hypertension when compared with patients without portal hypertension. However, no differences were observed between the two groups, when serum levels of γGT were evaluated (24). Similarly, in the present study differences were observed in serum levels of ALP between HS and splenectomized patients, but there were no differences in relation to levels of yGT. This finding might suggest that the elevation of ALP serum levels are related to portal hypertension, insofar as splenectomy, which reduces the portal flow, reduced levels of this enzyme (24). Probably, the elevation of yGT in schistosomiasis is not associated with portal hypertension but rather with advanced PPF.

In our study lower serum levels of bilirubins were also observed in splenectomized patients compared with HS patients. Similarly, Toledo et al. (2009)[24] also observed higher serum levels of bilirubins between schistosomal patients with portal hypertension when compared with patients without portal hypertension. The lowest levels of indirect bilirubin fraction in splenectomized patients could be due to the interruption of the hemolysis that occurs in the splenic parenchyma, due to hypersplenism (19). Furthermore, the lower levels of direct bilirubin fraction could be from the better capacity for synthesis of hepatocytes in virtue of the reduction of portal pressure in splenectomized patients.

In fact, some studies have revealed improved liver function tests in cirrhotic patients, including serum levels of albumin, after splenectomy (25-26). It is assumed that the improvement in the capacity for synthesis of hepatocytes after splenectomy arises from changes in hemodynamic conditions, with a reduction in mediators of inflammation (27). However, Ushitora et al., (2011)[28] did not find improvement in albumin serum levels in the late postoperative period of cirrhotic patients who had undergone splenectomy. It is worth remembering, however, that in our study schistosomal patients were evaluated, an illness in which the hepatocytes are preserved and the capacity for synthesis was not affected. In fact, the albumin serum levels in the two groups of patients evaluated were within the bounds of normality.

Another aspect should be pointed out with respect to the design of our study. In published studies of cirrhotic patients, the same patients were evaluated before and after splenectomy, with a short evaluation period after surgery. In our study, different patients were evaluated, with a long period after splenectomy, (average of 11.5 years).

Despite the capacity for synthesis of hepatocytes being conserved in patients with schistosomiasis, some patients in an advanced stage of the disease had hemostatic abnormalities and in the mechanisms of fibrinolysis (12-15).

Studies have shown changes in the PT, PTT and TT in HS patients and that these findings become more evident with the progression of this disease. The prolongation of PT, PTT and TT as well as hypofibrinogenemia has been reported in patients with schistosomiasis (13). In fact, in our study, the prolongation of the PT and PTT were observed in HS patients. Meanwhile, in splenectomized patients no abnormalities were found in PT and PTT, suggesting that these changes are due not only to the capacity for synthesis of hepatocytes, but also to the degree of portal hypertension. Furthermore, slightly higher fibrinogen levels were also found in the splenectomized patients when compared to the HS group, although this difference was not significant.

Changes in the levels of factors II, VII, IX, X, and protein C have also been reported in patients with schistosomiasis (13). Indeed, in our study lower levels of factors II, VII, VIII, IX, X, protein C were found in the HS group and levels close to normality in the splenectomized patients. It is assumed that the mechanisms responsible for the reduction of vitamin K in HS schistosomiasis patients are the reduction of hepatic synthesis or increased consumption of these coagulation factors, (13-14). As a result of our findings with regression of changes to the splenectomized patients, it is suggested that the reduction of portal pressure after splenectomy should improve the capacity of synthesis and reduce the consumption of the factors.

Another interesting finding of this study relates to lower levels of PAI-1 in the HS group, as described by El-Bassiouni.et al[15]. It is assumed that PAI-1 is partially synthesized by the liver and

partially by the endothelial cells (29-31). Thus, the higher level of PAI-1 in the splenectomized patients suggests that the reduction of portal pressure improves the generation of PAI-1, in both hepatocytes and endothelial cells.

Finally, this study suggests that changes occur in liver enzymes and components of hemostasis in HS schistosomiasis patients and that these changes are not found in the splenectomized patients. It is assumed that the liver abnormalities and hemostatic dysfunction in HS patients may be influenced by portal hypertension that occurs in schistosomiasis mansoni.

References

1. Gryseels B, Polman K, Clerinx K, Kestens L. Human schistosomiasis. Lancet 2006; **368**: 1106-1118.
2. Ross AGP, Bartley PB, Sleigh AC, *et al*. Schistosomiasis. N Engl J Med. 2002; **346**: 1212-1220.
3. Leite LAC, Pimenta Filho AA, Fonseca CSM, *et al*. Hemostatic Dysfunction is Increased in Patients with Hepatosplenic Schistosomiasis mansoni and Advanced Periportal Fibrosis, Plos Neglected Diseases. 2013; **18**: e2314.
4. Katz N, Peixoto SV. Critical analysis of the estimated number of schistosomiasis mansoni carriers in Brazil. Rev Soc Bras Med Trop. 2002; **33**: 303-308.
5. Andrade ZA. Schistosomal hepatopathy. Mem Inst Oswaldo Cruz 2004; **99**: 51–7.
6. Morais CNL, Souza JR, Melo WG, *et al*. Cytokine profile associated with chronic and acute human schistosomiasis mansoni. Mem. Inst. Oswaldo Cruz. 2008; **103**: 561-568.
7. Ferraz AAB, Albuquerque PC, Lopes EPA, *et al*. The influence of periportal (Pipestem) fibrosis on long term results of surgical treatment for schistosomotic portal hypertension. Arq Gastroenterol 2003; **101**: 183-186.
8. Leite LAC, Domingues ALC, Lopes EP, *et al*. Relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis. Rev Bras Hematol Hemoter, 2013; **35**: 332-336.
9. Denié C, Vachiery F. Elman A, *et al*. Systemic and splanchnic hemodynamic changes in patients with hepatic schistosomiasis. Liver International 1996; **16**: 309–312
10. De Cleva R, Herman P, D'albuquerque LA, *et al*. Pre and postoperative systemic hemodynamic evaluated in patients subjected to esophagogastric devascularization plus splenectomy and distal splenorenal shunt. A comparative study in schitosomal portal hypertension. World J Gastroenterol 2007; **13**: 5471-77.
11. Ferraz AAB, Lopes EP, Barros FM, *et al*. Esplenectomia com ligadura da veia gástrica esquerda e desvascularização de grande curvatura do estômago no tratamento da esquistossomose hepatoesplenomegalia. É necessária a escleroterapia endoscópica pós-operatória. Arq Gastroenterol 2001; **38**: 84-88.
12. Camacho-Lobato LC, Borges DR. Early liver dysfunction in schistosomiasis. Journal of hepatology 1998; **29**: 476-78.
13. Omran SA, Amin HM, El-Bassiouni NE, *et al*. Vitamin K dependent coagulation proteins in endemic hepatosplenomegaly in Egypt. J Clin Pathol 1993; **47**: 502-04.
14. Tanabe M. Haemostatic abnormalities in hepatosplenic schistomomiasis mansoni. Parasitol Int 2003; **52**: 351–359
15. El-Bassiouni NE, El-Bassiouni AE, Khayat HE, *et al*. Hyperfibrinolysis in hepatosplenic schistomiasis. J Clin Pathol 1996; **49**: 990-993.
16. Makdissi FF, Herman P, Machado MMC, *et al*. Trombose de veia porta após desconexão ázido-portal e esplenectomia em pacien-tes esquistossomóticos. Qual a real importância? Arq Gastroenterol. 2009; **46**:50-6.
17. Richter J, Domingues ALC, Barata CH, *et al*. Report of the Second Satellite Symposium on Ultrasound in Schistosomiasis. Mem Inst Oswaldo Cruz 2001: **96**; 151-156.
18. Dias HS, Domingues ALC, Cordeiro FTM, *et al*. Associating portal congestive gastropathy and hepatic fibrosis in hepatosplenic mansoni schistosomiasis, Acta tropica 2013; **126**:240-246.
19. Maia MD, Lopes EPA, Ferraz AAB, *et al*. Evaluation of splenomegaly in the hepatosplenic form of mansonic schistosomiasis. Acta Tropica 2007; **101**: 183-86.

20. Ferraz AA, Bacelar TS, Silveira MJ, *et al*. Surgical treatment of schistosomal portal hypertension. Int Surg. 2001; **86**:1-8.
21. Barreto VST. Alkaline phosphatase in schistosomiasis. Ann Inter Med 1971; 74: 450-1.
22. Amaral ACC, Köpke-Aguiar LA, Souza MRA, *et al*. Elevação da γ-glutamiltransferase sérica na hepatopatia esquistossomótica não se correlaciona com a carga parasitária e precede alterações ultrassonográficas. Arq Grastroenterol 2002; 39: 27-31.
23. Alves Jr A, Fontes DA, Melo VA, *et al*. Hipertensão Portal Esquistossomótica: influência do fluxo sanguíneo portal nos níveis séricos das enzimas hepaticas. Arq Gastroenterol 2003; **40**: 203-208.
24. Toledo CF, Carvente CT, Shigueoka DC, Borges DR. Endothelial Markers in Schistosomiasis Patients With or Without Portal hypertension. Dig Dis Sci 2009; **51**: 1331-36.
25. Kedia S, Goyal R, Mangla V, *et al*. Splenectomy in cirrhosis with hypersplenism: improvement in cytopenias, Child's status and institution of specific treatment for hepatitis C with success Ann Hepatol. 2012; **11**: 921-929.
26. Ikegami T, Shimada M, Imura S. Recent role of splenectomy in chronic hepatic disorders. Hepatology Research 2008; **38**: 1159–1171.
27. Jiang H, Meng F, Wei Li, *et al*. Splenectomy ameliorates acute multiple organ damage induced by liver warm ischemia reperfusion in rats. Surgey 2007; **38**: 32–39.
28. Ushitora Y, Tashiro H, Takahashi S, et al. Splenectomy in chronic hepatic disorders: portal vein thrombosis and improvement of liver function. Dig Surg. 2011; **28**: 9-14.
29. Omran SA, Hussein NA, Mohamed AA, *et al*. Fibrinolysis and bleeding tendency in patients with hepatosplenic schistomiasis. J Clin Pathol 1990 **43**: 476-78.
30. Sprengers ED, Prince HGM, Kooistra T, Hinsbergh VWM. Inhibition of plasminogen activators by conditioned medium of human hepatocytes and hepatoma cell line HepG2. J Lab Clin Med 1985; **105**: 751-758.
31. Fujii S, Lucore CL, Sobel BE. Induction of endothelial cell synthesis of plasminogen activator inhibitor by t-PA. Circulation 1989; **80** (Suppl 2): 111-115.

Table 1 - Demographic characteristics and ultrasound parameters of hepatosplenic form and splenectomized schistosomiasis patients.

Characteristics	Schistosomiasis patients	
	Hepatosplenic	Splenectomized
Number of patients	55	45
Age	50.20 ± 1.86	50.19 ± 1.41
Gender		
Male	50.9%	37.8%
Female	49.1%	62.3%
Diameter portal vein (cm)	1.28 ± 0.04	0.96 ± 0.26[a]
Fibrosis pattern		
D	30.9%	15.6%
E	54.6%	73.3%
F	14.5%	11.1%

Values were expressed in mean±Standard Error (SE); [a]$p < 0.05$

Table 2 - Liver function tests in hepatosplenic form or splenectomized schistosomiasis patients.

Liver Parameters	Schistosomiasis Patients	
	Hepatosplenic	Splenectomized
Number of patients	55	45
AST/ULN (IU/L)	1.58 ± 0.14	1.45 ± 0.09
ALT/ULN (IU/L)	1.40 ± 0.16	1.29 ± 0.12
ALP/ULN (IU/L)	1.10 ± 0.08	0.88 ± 0.06[a]
γ-GT/ULN (IU/L)	3.11 ± 0.37	3.35 ± 0.42
Albumin (g/L)	39.8 ± 0.79	40.4 ± 0.99
Total bilirubin (mg/L)	12.3 ± 1.22	8.29 ± 0.39[a]
Direct bilirubin (mgl/L)	5.07 ± 0.08	3.34 ± 0.03[a]
Indirect bilirubin (mg/L)	7.12 ± 0.70	4.96 ± 0.33[a]

Values were expressed in mean±Standard Error (SE); [a]$p < 0.05$, ULN, Upper Limit of Normal

Table 3 - Coagulation and fibrinolytic parameters in hepatosplenic form or splenectomized schistosomiasis patients.

Coagulation tests		Schistosomiasis groups	
	Normal range	Hepatosplenic	Splenectomized
Number of patients		55	45
Platelets Count (1000/mm^3)	150-400	128.4 ± 12.0	254.1 ± 10.4[a]
PT (Sec)	11.5-13	19.3 ± 0.63	13.8 ± 0.63 [a]
TT (Sec)	10-15	13.7 ± 0.21	13.9 ± 0.26
PTT (Sec)	26-43	37.9 ± 1.47	26.9 ± 0.16 [a]
Fibrinogen (g/L)	1.8-4.0	2.62 ± 0.10	2.82 ± 1.2.1
Factor II (U/mL)	0.5-1.5	0.66 ± 0.01	0.70 ± 0.01 [a]
Factor VII (U/mL)	0.5-1.5	0.49 ± 0.02	0.65 ± 0.02 [a]
Factor VIII (U/mL)	0.5-1.5	0.90 ± 0.04	1.06 ± 0.06 [a]
Factor IX (U/mL)	0.5-1.5	0.69 ± 0.03	0.83 ± 0.03[a]
Factor X (U/mL)	0.5-1.5	0.61 ± 0.03	0.77 ± 0.03[a]
Protein C (U/mL)	0.4-1.6	0.63 ± 0.02	0.75 ± 0.04[a]
Antithrombin IIa (U/mL)	0.75-1.25	0.92 ± 0.03	0.964 ± 0.04

Values were expressed in mean±Standard Error (SE), [a]$p < 0.05$

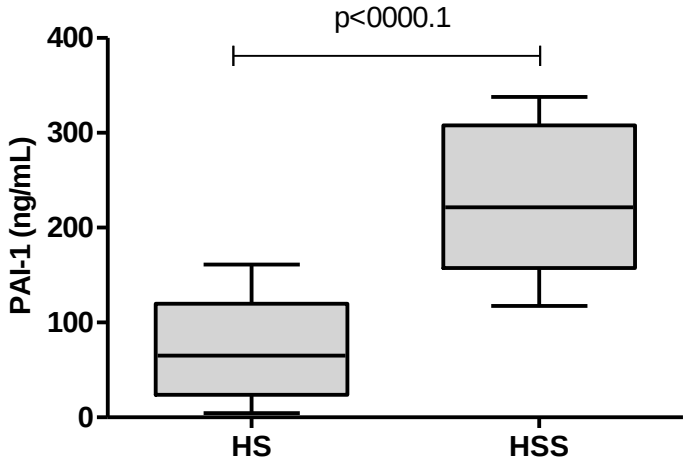

Figure 1 – Box Plot of plasma levels of PAI-1 in hepatosplenic (HS) and splenectomized (HSS) schistosomiasis patients. The solid horizontal lines show PAI-1 median values (51.9 and 204.1 ng/mL); Mann Whitney test.

CAPÍTULO IV

Portal Vein Thrombosis Associated with Protein C Deficiency in Hepatosplenic Schistosomiais: A Case Report.

Impact Factor: 2.439

Portal Vein Thrombosis Associated with Protein C Deficiency in Hepatosplenic Schistosomiais: A Case Report.

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Competing interests: None.

Funding: The authors received financial support for this study from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/ BRAZIL.

The funders had no role in study design, data collection and analysis, in the decision to publish, or in preparation of the manuscript.

ABSTRACT

Portal vein thrombosis is considered a rare event in the course of hepatosplenic schistosomiasis mansoni. Portal vein thrombosis may be the result of an impairment of the portal blood flow or may be associated with acquired or inherited thrombophilic factors. We report on a 63-year-old woman with a 25-year history of hepatosplenic schistosomiasis who developed portal hypertension and thrombocytopenia as a result of hypersplenism. She had recurrent bleedings. After the diagnosis of hepatosplenic schistosomiasis, splenomegaly, advanced periportal fibrosis and portal vein thrombosis were detected upon ultrasound examination. Hematological tests also revealed low levels of protein C (43.3%) and high levels of factor VIII (183.1%). The pathogenesis of portal vein thrombosis remains unclear in some patients with schistosomiasis mansoni. Therefore for these patients we recommend that an early clinical and hemostatic investigation should be done in order to evaluate the risk of portal vein thrombosis and to avoid further complications.

Schistosomiasis affects more than 200 million people around the world.[1] In northeastern Brazil, approximately 3 to 7% of the patients infected by *Schistosoma mansoni* develop the most severe form of the disease, the hepatosplenic form (HS).[2] This form is characterized by hepatic fibrosis around the portal branches caused by granulomatous reaction to the eggs of the parasite, periportal fibrosis, portal hypertension, and splenomegaly. In many cases, portal hypertension induces formation of esophageal varices that can rupture and cause episodes of upper gastrointestinal bleeding.[3-4] However, the architecture and function of the liver are still preserved, to the extent that the parasite does not destroy the hepatocyte and fibrosis occurs at a pre-sinusoidal level.[4]

On the other hand, some patients develop hemostatic disorders, which can result in portal vein thrombosis (PVT), described in about 5% of the patients with HS schistosomiasis.[5] Splenectomy with left gastric vein ligation and esophagogastric disconnection is often used for correction of portal hypertension and prevention of new episodes of gastrointestinal bleeding, however, the prevalence of PVT increases significantly, to around 13.3 to 53.2% after this surgical procedure.[6]

PVT can feature total or partial occlusion of the portal vein and portal flow reduction, and may be detected during routine ultrasound examination, although the gold standard for diagnosis is portography.[7-10] PVT can be related to various clinical conditions, including cirrhosis, neoplasia, thrombophilia (prothrombinic mutation G20210A, Factor V Leiden), inflammatory and infectious diseases, trauma, myeloproliferative disorders, and surgical interventions such as splenectomy.[11-12]

In cirrhosis, hemostatic changes may involve both the procoagulant factors dependent on vitamin K such as anticoagulants, due to imbalances between levels of protein C and coagulation factors, leading to the emergence of thrombosis.[13]

The aim of this present study was to report a case of partial portal vein thrombosis resulting from protein C deficiency, which extended to the spleno-portal axis in a patient with hepatosplenic schistosomiasis, including thrombocytopenia and hemorrhagic manifestations.

CASE REPORT

Female patient, 63 years old, born and residing in a municipality endemic for *S. mansoni*, bearer of HS schistosomiasis. The patient arrived at the Gastroenterology Clinic of the “Hospital das Clínicas, Federal University of Pernambuco (UFPE) in March 2005 after an episode of upper gastrointestinal bleeding. Digestive endoscopy showed varicose strands of thin and medium caliber in the lower third of the esophagus and gastric fornix, with the presence of tight cavitary knobs, in addition to light intensity congestive gastropathy with antral erosions. Sclerotherapy of varices was carried out with Ethamolin. After 2 years, the patient was hospitalized due to a new episode of upper gastrointestinal bleeding with arterial hypotension and collapse of hematocrit and hemoglobin (Hct, 22.7%/Hgb 7.4 g/dL), receiving two units of blood and being subjected to elastic ligature of varices. The patient denied use of ethanol,

tobacco, history of neoplasms or thrombosis in the family, and did not mention contact with contaminated water after specific treatment with praziquantel (50 mg/Kg). There was no evidence of diabetes, hypertension or dyslipidemia and the patient also denied hormone replacement therapy and use of hepatotoxic drugs. Upon physical examination, the patient was found to have a body mass index of 22.2 kg/m^2, a liver 3 cm below the xiphoid process and a spleen 5 cm below the left costal edge. The sonogram showed a liver with a diminished right lobe, enlarged left lobe, and advanced periportal fibrosis, (pattern E), a portal vein with 1.80 cm diameter and splenic vein of 1.10 cm, longitudinal diameter of spleen 16.3 cm and absence of ascites. On this occasion the portal vein flow was hepatopetal and thrombosis was not detected. The laboratory examinations showed serum levels of bilirubins (Total, Direct and Indirect), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γGT), alkaline phosphatase (ALP) and albumin were normal. The patient presented prothrombin index, INR of 1.37 and an activated thromboplastin time (PTT) of 29 seconds (25 to 45 seconds). The Complete Blood Count showed the following: RBC 4.92x10^{12}/L (4.00 to 5.20x10^{12}/L), Hb 9.1 g/dL (12 to 16 g/dL), Hct 29.3% (36 to 46%), WBC 1.3x10^9/L (3.8 to 10.0x10^9/L), platelets 36x10^9/L (150 to 400x10^9/L). Tests showed negative serology for hepatitis B, C and HIV, anti-DNA negative and TSH of 2.43 (0.4 to 4.1 µM/mL) and T3 and T4 were normal.

Propanolol at 40 mg/day was initiated with outpatient follow-up. In August 2012, control ultrasound showed the same hepatic alterations of the previous examination, with partial thrombosis of the portal vein detected (Figure 1), which extended until the splenic vein. An increased portal vein was noticed whose diameter measured 2.04 cm, with a flow rate of 14.1 cm/s, showing partial thrombus; the diameter of the splenic vein was measured at 1.27 cm also with thrombus; the spleen had a longitudinal diameter of 16.2 cm (Figure 2). Collateral splenorenal circulation was also noticed. New complementary examinations were requested: RBC 4.92X10^{12}/L, Hb 7.7 g/dL, Hct 38.9%, WBC 2.30X10^9/L, platelets 42x10^9/L, negative rheumatoid factor and negative antinuclear factor, iron 48.1 µg/dL (37 to 145 µg/dL) and ferritin serum 152.4 ng/mL (13 to 150 ng/mL). Liver function was still preserved (normal serum levels of bilirubin, AST, ALT, γGT, ALP and albumin). In addition, examinations were held for investigation of thrombophilia: INR 1.38, PTT 31.8 seconds, fibrinogen concentration 2.4 g/L (1.5 to 3.7 g/L), TT 13 seconds (9 to 15 seconds), concentrations of factors [II 94.5%, VII 80.7%, VIII 183.1%, XI 112%, X 88.1%, (50 to 150%), D-dimer 995.4 ng/mL (up to 500 ng/mL), protein C 43.3% (70 to 140%), protein S 77.3% (60.1 to 113.6%), antithrombin IIa 90.2% (75 to 125%), von Willebrand factor (Fvw) 122% (50 to 150%), with aggregation to ristocetin 200%, proving negative for von Willebrand disease. The search for the factor V Leiden mutation, prothrombin G20210A and methylene C677T were performed by PCR/RFLP, being all negative. The patient continues to be accompanied in the Gastroenterology Service/UFPE, showing distributed bruises (Figure 3) and use of propanolol of 40 mg/day.

DISCUSSION

In most cases HS schistosomiasis develops with preserved liver function, though some patients show hemorrhagic and thrombotic manifestations.[5,14] The emergence of thrombosis is determined by changes in any one of the three components of Virchow's triad: venous stasis, endothelial injury and hypercoagulability.[13]

In the present report, the patient presented thrombosis of the portal vein that extended to the splenic vein, which could be related to changes in endothelium caused by increased mechanical stress on flow. It is worth noting also the greater generation of thrombin, and protein C deficiency, described in patients with HS schistosomiasis with advanced patterns of periportal fibrosis.[15] In addition, the patient also showed increase of factor VIII, described as a risk factor for thrombosis in patients with chronic liver diseases.

However, these data are controversial, because most patients with HS schistosomiasis display portal hypertension in different degrees even with preserved liver function and yet do not frequently exhibit thrombosis.[4,14]

In a recent study, Leite et al., 2013, demonstrated that HS patients exhibited abnormalities in hepatic enzymes and blood-clotting proteins, mainly in serum levels of γGT and protein C. The authors also found that the reduction in protein C was more evident in patients with schistosomiasis with more advanced periportal fibrosis. In fact, in schistosomiasis post-necrotic scars have already been described resulting from focal ischemic necrosis of liver parenchyma after episodes of upper gastrointestinal bleeding.[4,15] This leads us to believe that changes may occur in the capacity for synthesis of hepatocytes, mainly in patients who display advanced hepatic fibrosis or after episodes of upper gastrointestinal bleeding.

Some studies show patients with compensated and uncompensated HS schistosomiasis patients showed high levels of D-dimer, indicating persistent activation of the coagulation system and increased thrombin generation.[14,16] This finding was also found in the present report, and may be associated with greater degradation of fibrin and hyperfibrinolysis, which could aggravate episodes of upper gastrointestinal bleeding and propitiate bruising, as shown by the patient.

Correia et al, 2009, described that patients with HS schistosomiasis had high levels of FvW and that this finding can promote stabilization of microaggregate platelets and can prevent haemorrhagic manifestations in cases of thrombocytopenia. However, in this case, the patient exhibited advanced hepatosplenia, marked thrombocytopenia, but elevated levels of FvW were not found in this study.[17]

Some studies have shown that the main findings associated with thrombotic events are the elevation of plasma levels of factor VIII and concomitant decrease in protein C, with these the most common findings in final stages of liver disease.[13]

In this case, the patient exhibited marked thrombocytopenia due to hypersplenism and portal hypertension with upper gastrointestinal bleeding, the latter being related to rupture of varices. The levels of FvW did not result in protection against bleeding episodes.

Studies by Tripodi&Mannucci 2011 have postulated that the concomitant reduction of procoagulant and anticoagulant factors can explain why patients with chronic liver disease are protected from thrombotic events and that the imbalance between the levels of factor VIII and protein C (decreased protein C and increased factor VIII) indicates a state of hypercoagulability. In the present case a deficiency of

protein C was detected, being one of the main causes of thrombosis in patients with liver disease.[13]

It is probable that protein C deficiency associated with both the reduction of portal blood flow and the increase in factor VIII levels have been responsible for portal vein thrombosis in this patient. It is suggested that future studies be conducted, using a larger number of patients with a protocol which includes tracking of portal vein thrombosis accomplished by Doppler ultrasound and tracking levels of coagulation factors to explain the genesis of hemorrhagic and thrombotic events in patients with hepatosplenic schistosomiasis

REFERENCES

1 **Ross AGP**, Bartley PB, Sleigh AC, *et al*. Schistosomiasis. N Engl J Med 2002; 346: 1212–1220.

2 **Maia MD**, Lopes EPA, Ferraz AAB, *et al*. Evaluation of splenomegaly in the hepatosplenic form of mansonic schistomiasis. Acta Tropica 2007; 101: 183-86.

3 **Andrade Z**. Schistosomal hepatopathy. Mem Inst Oswaldo Cruz 2004; 99: 51-57.

4 **Dias HS**, Domingues ALC Cordeiro FTM, *et al*. Associating portal congestive gastropathy and hepatic fibrosis in hepatosplenic mansoni schistosomiasis, Acta tropica 2013; 126:240-246.

5 **Lambertucci RJ**, Resende V, Voieta I. Portal vein thrombosis in a patient with hepatosplenic schistosomiasis mansoni. Rev Bras Med Trop 2009; 24: 235-236.

6 **Ferraz, AAB**, Albuquerque, PC, Lopes, EP, *et al*. The influence of periportal (pipestem) fibrosis on long term results of surgical treatment for schistosomotic portal hypertension. Arq Gastroenterol 2003; 10: 4-10.

7 **Choi Bo K,** Yang SH, Suh KH, *et al*. A Case of Portal Vein Thrombosis by Protein C and Protein S Deficiency Completely Recanalized by Anticoagulation Therapy. Chonnam Med J 2011; 47: 185-188.

8 **Machado MM,** Rosa ACF, Mota OM, *et al*. Aspectos Ultrassonográficos da trombose da veia porta. Rad Bras 2016; 39: 151-155.

9 **Amitrano L,** Guardascione MA, Brancaccio V**,** *et al*. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. J Hepatol 2004; 40: 736-741.

10 **Ramos R,** Park Y, Shazad G, *et al*. Cavernous Transformation of Portal Vein Secondary to Portal Vein Thrombosis: A Case report. J Clin Med Res 2011; 4: 81-84.

11 **Dentali F,** Galli M, Gainni M, *et al*. Inherited thrombophilic abnormalities and risk of portal vein thrombosis. Thromb Haemost 2008; 99: 675-682.

12 **Fimognari FL**, Violi F. Portal vein thrombosis in liver cirrhosis. Intern Emerg Med 2008; 3: 213-218.

13 **Tripodi A**, Mannucci PM. The Coagulopathy of Chronic Liver Disease. N Engl J Med 2011; 365: 147-56.

14 **Camacho-Lobato L**, Borges DR. Early liver dysfuction in schistosomiasis. J Hepatol 1998; 29: 233–240.

15 **Leite LAC**, Pimenta Filho AA, Fonseca CSM, *et al*. PLOS Neglected Tropical Diseases 2013; 7: 1-5.

16 **Omran SA,** Amin HM, El-Bassiouni NE, *et al*. Vitamin K dependent coagulation proteins in endemic hepatosplenomegaly in Egypt. J Clin Pathol 1994; 47: 502–504.

17 **Correia MCB**, Domingues ALC, Lacerda HR, *et al*. Platelet function and the von Willebrand factor antigen in the hepatosplenic form of schistosomiasis mansoni. The Royal Society of Tropical Medicine and Hygiene . 2009; 103: 1053-1058.

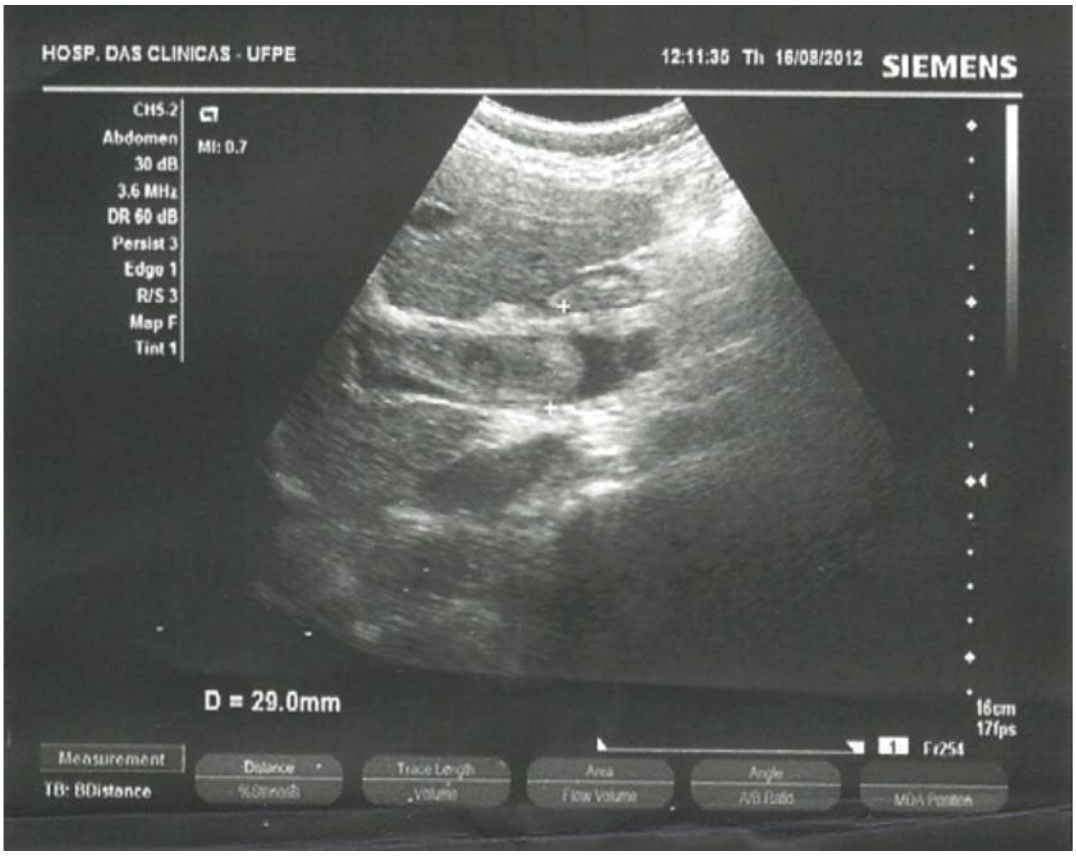

Figure 1 – Portal vein thrombosis visualized by ultrasound of abdomen in a patient with hepatosplenic schistosomiasis mansoni.

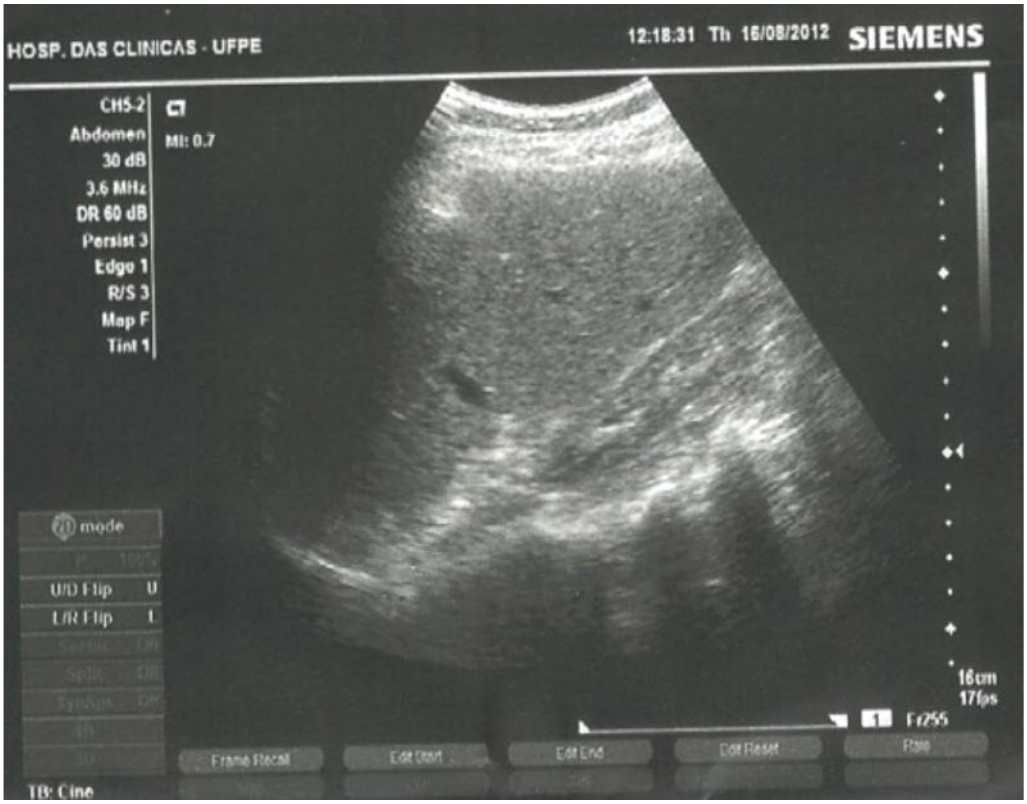

Figure 2 – Massive splenomegaly as seen through ultrasound of the abdomen in a patient with hepatosplenic schistosomiasis mansoni.

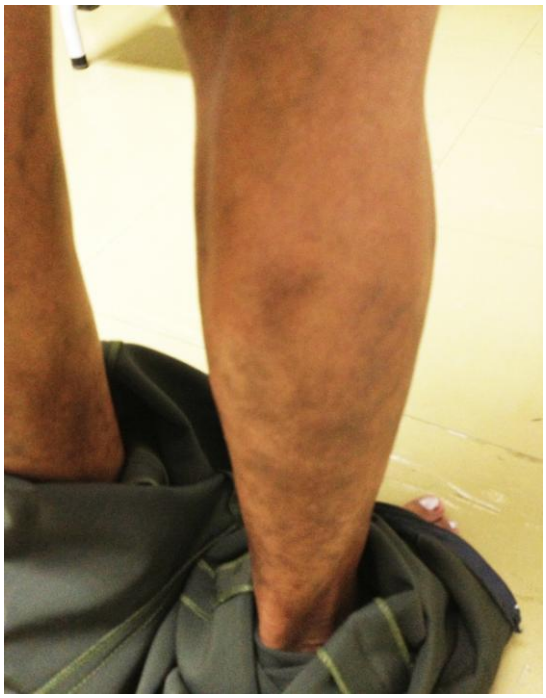

Figure 3 – Patient with hepatosplenic schistosomiasis mansoni, displaying haemorragic manifestations (ecchymosis).

7. CONCLUSÕES

✓ A disfunção hemostática em pacientes com esquistossomose na forma HE está associada à fibrose periportal, gerando redução acentuada dos fatores da coagulação, da proteína C, e aumento dos níveis de DD, sendo esse o primeiro estudo a demonstrar que a proteína C e pode ser um marcador de função hepática e de padrões de fibrose avançada. O estudo também mostrou que existe associação entre as hepáticas, às proteínas de coagulação e os padrões de fibrose periportal avançada.

✓ A hipertensão portal tem um papel relevante sobre os testes da coagulação e da fibrinólise, pois a esplenectomia provoca melhora significativa nos testes hemostáticos (INR, TTP, fatores II, VII, VIII, IX, X e proteína C).

✓ Este estudo descreveu um raro caso de trombose de veia porta associado à deficiência de proteína C e sangramentos em uma paciente com esquistossomose na foram HE.

✓ A presente tese leva a crer que os testes de coagulação são marcadores precoces de disfunção hepática, principalmente o fator VII e a proteína C. Além disso, pode existir status de coagulopatias subclínicas em pacientes esquistossomóticos, devendo ser acrescidos aos testes de rotina alguns marcadores da coagulação, tanto em pacientes ambulatoriais internados como também antes de procedimentos cirúrgicos.

8. PERSPECTIVAS

Novos estudos utilizando grupos de pacientes esquistossomóticos antes e após a esplenectomia, ou mesmo em pacientes com e sem uso de betabloqueadores (propanolol), devem ser desenvolvidos para investigar a influência da hipertensão portal sobre os níveis plasmáticos das proteínas da coagulação. Muitos eventos trombóticos em pacientes com esquistossomose permanecem sem explicação, pois estes pacientes não exibem *status* de hipercoagulabilidade, sendo necessários novos estudos que possibilitem analisar um grande número de pacientes esquistossomóticos antes e após a esplenectomia. É de suma importância investigar os parâmetros fibrinolíticos em pacientes com esquistossomose com hemorragia digestiva recente para verificar se existe indicação de terapia antifibrinolítica nos pacientes com esquistossomose.

9. APÊNDICE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (T.C.L.E.)

Eu,_____, certifico, através deste termo, que concordo em participar da pesquisa intitulada "**ESTUDO DA COAGULAÇÃO E FIBRINÓLISE EM PACIENTES PORTADORES DE ESQUISTOSSOMOSE HEPATOESPLÊNICA E SUA RELAÇÃO COM APOLIPOPROTEÍNAS PLASMÁTICAS**". Para tanto doei 10 ml do meu sangue, obtido por punção venosa, para a determinação laboratorial de parâmetros bioquímicos, como perfil lipídico e lipoprotéico e determinação dos níveis das proteínas de coagulação e enzimas fibrinolíticas. Asseguro que me encontro em jejum de no mínimo 12 horas. Também fui informado dos possíveis riscos da coleta sanguínea, que são o aparecimento de pequenas equimoses e desconforto no local, após a coleta. Entretanto, tomando todos os cuidados necessários as chances de acontecer este tipo de complicação são mínimas.

Relataram-me também que todo o material utilizado para a coleta sangüínea será descartável e estéril, não apresentando riscos para contaminação e que serão descartados em recipientes impermeáveis, de paredes rígidas e devidamente identificados como resíduos de risco biológico e encaminhados à empresa especializada em coleta e processamento de resíduos de serviços de saúde (SERQUIP) que presta este tipo de serviço ao Departamento de Bioquímica/CCB/UFPE, conforme especificações da RDC nº 306, de 7/12/2004 (ANVISA).

Foi explicado que a minha participação na presente pesquisa é fundamental para o esclarecimento da fisiopatologia da esquistossomose e sua relação com a coagulação e apolipoproteínas, porém não é obrigatória e eu posso retirar-me a qualquer momento do estudo, desde que assim eu deseje. A minha recusa não trará nenhum prejuízo em minha relação com os pesquisadores ou com a Instituição. Terei livre acesso aos resultados dos meus exames bioquímicos, sem qualquer geração de ônus para mim. Também não estou recebendo nenhum tipo de prêmio ou remuneração para participar da presente pesquisa. Sou apenas um participante voluntário. Sei que as informações serão publicadas, mas apenas de modo estatístico, com minha identidade sendo preservada. Declaro, portanto, que entendi os objetivos, riscos e benefícios de minha participação, e concordo,

voluntariamente, em participar do presente estudo, e que estou recebendo uma cópia deste termo de consentimento livre e esclarecido.

Nome e Endereço do Pesquisador Responsável: (Obrigatório)

VERA LÚCIA DE MENEZES LIMA

Universidade Federal de Pernambuco – UFPE

Centro de Ciências Biológicas – CCB / Departamento de Bioquímica

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APÊNDICE II

CONSIDERAÇÕES SOBRE OS RESULTADOS DA TESE

EM RELAÇÃO AOS TESTES DE FUNÇÃO HEPÁTICA

1.1 – Os indivíduos portadores de esquistossomose na forma hepatoesplênica (HE) mostraram que os testes de função hepática (AST, ALT, γ-GT, FA, bilirrubina total) foram maiores que nos controles e que houve menor média de albumina em relação aos controles normais.

1.2 – Os níveis séricos de AST, ALT e γ-GT não foram diferentes entre os pacientes de forma HE e forma HE esplenectomizados. Os pacientes da forma HE apresentaram aumento significante nos níveis de FA, bilirrubina total e frações, e diminuição da média de albumina em relação aos pacientes esplenectomizados.

EM RELAÇÃO AOS TESTES DE COAGULAÇÃO

1.3 - Os pacientes com esquistossomose na forma HE mostraram aumento significativo do INR, TTP, TP, e menor média de plaquetas que os controles normais. Todos os fatores (II, VII, VIII, IX e X) estiveram significativamente mais baixos que os controles normais.

Os pacientes com esquistossomose na forma HE esplenectomizados apresentaram maior média de plaquetas, valores inferiores de INR, TTP e aumento dos níveis de fatores (II, VII, VIII, IX e X) e fibrinogênio. Não houve diferença entre os pacientes esplenectomizados ou não em relação ao tempo de trombina.

2 − EM RELAÇÃO AOS NÍVEIS DE PROTEÍNAS ANTICOAGULANTES NATURAIS

2.1– A atividade da proteína C foi menor nos pacientes esquistossomóticos na forma HE em relação aos pacientes esplenectomizados e controles.

2.2 - Os níveis de antitrombina IIa foram menores nos pacientes com esquistossomose na forma HE em relação aos controles normais e também foram menores quando comparados os pacientes de doença hepática crônica mista com os pacientes esquistossomóticos na forma HE. Contudo, não houve diferença entre os níveis de antitrombina IIa entre os pacientes HE e os esplenectomizados.

3 − RELAÇÃO DOS TESTES DE COAGULAÇÃO COM O PADRÃO DE FIBROSE

3.1- Houve diferença significante entre os testes de coagulação e entre os grupos de fibrose D (central) e E+F (avançada + muito avançada), em relação aos controles normais. Apenas a proteína C mostrou diferença entre os dois grupos de fibrose (D VS E+F).

3.2– Os níveis de D-dímero foram significantemente maiores no grupo de pacientes com a forma HE (grupos de fibrose D e E+F), em relação aos controles normais.

4 – EM RELAÇÃO AO SISTEMA FIBRINOLÍTICO

4.1– Os níveis de fibrinogênio e PAI-1 foram menores no grupo de pacientes com esquistossomose na forma HE em relação ao grupo controle. Não houve diferença significante nos níveis de t-PA e TAFI entre os pacientes com a forma HE e controles.

4.2– Não houve diferença significante entre os níveis de t-PA e TAFI entre os pacientes HE e esplenectomizados.

APÊNDICE III

NORMAS DE SUBMISSÃO DO ARTIGO 3 – LIVER INTERNATIONAL

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APÊNDICE IV

NORMAS DE SUBMISSÃO DO ARTIGO 3 – J CLIN PATHOL

Instructions for authors

For general BMJ guidelines please follow the links below.

Manuscript formatting
Editorial policies
Patient consent forms
Licence forms
Peer review process
Online First process

Editorial policy

The Journal of Clinical Pathology (JCP) is committed to the advancement of all disciplines within the broader remit of human pathology. This also encompasses molecular biology and its applications in the understanding of human biology and pathology. The journal is intended to have world-wide readership and will publish articles that have a wide appeal even though they are regionally based.

Issues with a narrower restricted focus may be submitted as Letters to the Editor or as correspondence. JCP wishes to publish cutting edge, original clinical and laboratory-based articles, especially those with a clear clinical relevance. Provision of an educational platform for trainees, scientists and pathologists is an important function and aim of the journal. As such, state of the art reviews, viewpoints and editorials will be published.

The editorial team wishes to produce a balanced, informative and meaningful journal that is sensitive to the needs of its readership and the specialty at large, as well as being in tune with contemporary issues.

In pursuit of these goals we wish to publish work that is ethical (morally and scientifically), of a high quality and governed by a fair, independent peer review system.

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Article types and word counts

Original articles
Short reports
Reviews
Best Practice
My Approach / Demystified
Leading articles / Editorials
Letter to the Editor / Correspondence
eLetter correspondence
Multiple Choice Questions (MCQs)
Supplements

The word count excludes the title page, abstract, tables, acknowledgements and contributions and the references.

Abbreviations and symbols must be standard and SI units used throughout, except for blood pressure values which are reported in mm Hg.

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Original articles

Original articles should report original research of relevance to the understanding and practice of clinical pathology. They should be written in the standard form: abstract; introduction; methods; and discussion.

The journal uses a structured form of abstract in the interests of clarity. This should be short (no more than 250 words) and include four headings:

Aims - the main purpose of the study
Methods - what was done, and with what material
Results - the most important results illustrated by numerical data but not p values
Conclusions - the implications and relevance of the results

Authors of original articles are required to comply with one of the appropriate reporting guidelines endorsed by the EQUATOR Network. The following are the most commonly used guidelines for this journal. Authors are expected to submit the checklist that is most appropriate for their manuscript type:

Experimental studies - CONSORT Statement
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Word count: up to 2000 words.
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We are looking for diagnostic algorithms, investigative trees and/or any other useful hint(s) that will facilitate making the best/right diagnosis. These can include molecular techniques which may not be within the remit of every laboratory but certainly something that is doable.

Word count: between 2500 and 3000 words.
Abstract: up to 250 words.
Illustrations: at editorial discretion.
References: up to 150.

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Word count: up to 900 words.
Abstract: Not required.
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eLetter correspondence

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The MCQs set must contain at least 5 questions

Each question must have 5 possible answers, with only *one* answer being correct (the correct answer must be marked with an asterisk)

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ANEXOS I

Scientific Comments
DOI: 10.5581/1516-8484.20130114

Comment on: the relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis

Autores: Montenegro, Silvia Maria Lucena

Rev. Bras. Hematol. Hemoter. vol.35 no.5 São José do Rio Preto 2013

http://dx.doi.org/10.5581/1516-8484.20130114

REVISTA BRASILEIRA DE HEMATOLOGIA E HEMOTERAPIA

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Impact Factor: 0.27

Comment on: the relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis

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The aim of the authors of the paper 'Relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis' published in this edition of the *Revista Brasileira de Hematologia e Hemoterapia* was to evaluate hematological and hemostatic abnormalities in patients with the severe form of schistosomiasis and its possible association with splenomegaly and portal hypertension[1]. For this purpose a prospective study was performed of 55 compensated hepatosplenic schistosomiasis patients previously treated with praziquantel. All patients were outpatients of the Gastrointestinal Service of the *Hospital das Clínicas* of the *Universidade Federal de Pernambuco* (UFPE), Recife, Brazil, during the period 2010-2012. Thirty healthy individuals were selected as a control group. An abdominal ultrasound was performed in all patients and the Niamey protocols were used to measure the longitudinal diameter of the spleen and to classify the pattern of fibrosis. Moreover, routine liver tests were performed including albumin, aminotransferases (AST, ALT), alkaline phosphatase (ALP) and gamma glutamyl-transferase (γ-GT). A complete blood count and hemostatic tests such as prothrombin time/international normalization ratio (PT/INR), partial thromboplastin time (PTT), fibrinogen and D-dimer were carried out.

The authors observed that patients showed predominance of advanced pattern fibrosis. Furthermore, the routine liver and hemostatic tests had increased values compared to the control group with the exception of the level of fibrinogen. There were also high frequencies of upper gastrointestinal bleeding in patients (34%) and thrombocytopenia (83%); 36.5% had anemia and 47% presented leukopenia. An inverse correlation was found between the longitudinal diameter of the spleen and the platelet count.

The development of gastro-esophageal varices is a common complication of portal hypertension and bleeding from varices is a frequent cause of mortality and morbidity[2,3]. As the platelet count is a commonly available parameter and measurement of the spleen bipolar diameter has a high reproducibility in abdominal ultrasound studies[4,5], their use might be of help in the clinical management of patients with *Schistosoma mansoni* infection and suspected esophageal varices in endemic areas.

According to some authors[6,7], increases in the ALT and AST levels occur with liver cell damage, but in *S. mansoni* infections this type of injury is not commonly observed. On the other hand, the most important cause of increases in γ-GT is the chronic stimulation of the microsomal fraction of hepatocytes and the presence of cholestasis[8]. According to Martins & Borges, chronic stimulation of the microsomal fraction of hepatocytes occurs only in patients with the hepatosplenic form of schistosomiasis. Some authors[8,9] proposed that changes in the biliary tree, due to fibrosis of the portal space, may be the anatomical substrate for the increases in the ALP and γ-GT levels in patients with the hepatosplenic form of schistosomiasis.

In the literature, there is a tendency to explain hematologic changes related to portal hypertension as being due to hypersplenism just through observing hematological laboratory values[10,11]. It is noteworthy that pancytopenia in portal hypertension due to schistosomiasis is caused by intra-splenic blood stasis related to difficult venous drainage to the liver. Symmers fibrosis in hepatosplenic schistosomiasis does not interfere in the function of these organs. Therefore, there is doubt regarding the term hypersplenism which is perhaps confused with the term storage. In the case of splenomegaly schistosomiasis, cytopenia may be caused by the increase in splenic storage and not by a mononuclear phagocytic system disorder[11,12].

Apart from these considerations, there is the difficulty of explaining why the very low platelet and leukocyte counts found in patients with portal hypertension and schistosomiasis are not accompanied by clinical symptoms[11,13].

On the other hand, coagulation disorders are frequently observed in patients with schistosomiasis even though the pathophysiology for this has not yet been established. In patients with advanced hepatosplenic schistosomiasis, high levels of the pro-inflammatory cytokines, interleukin-1 alpha (IL-1α) and tumor necrosis factor alpha (TNF-α) and lipopolysaccharide (LPS) have been detected in the sera[14]. It is therefore assumed that a tissue factor procoagulant expressed by endothelial cells because of stimulation by these agents

Conflict-of-interest disclosure:
The author declares no competing financial interest

Submitted: 7/19/2013
Accepted: 8/7/2013

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DOI: 10.5581/1516-8484.20130114

may participate in the activation of the extrinsic coagulation cascade[14]. Accordingly, it may be possible that host immune responses to schistosome eggs participate in not only granuloma formation and tissue fibrosis, but also in the development of hemostatic abnormalities in schistosomiasis mansoni[14].

References

1. Leite LA, Domingues AL, Lopes EP, Ferreira RC, Pimenta Filho AA, Fonseca CS, et al. Relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis. Rev Bras Hematol Hemoter. Ahead of print.
2. Agha A, Abdulhadi MM, Marenco S, Bella A, Alsaudi D, El-Haddad A, et al. Use of the platelet count/spleen diameter ratio for the noninvasive diagnosis of esophageal varices in patients with schistosomiasis. Saudi J Gastroenterol. 2011;17(5):307-11.
3. De Franchis R, Dell'Era A, Primignani M. Diagnosis and monitoring of portal hypertension. Dig Liver Dis. 2008;40(5):312-7.
4. O'Donohue J, Ng C, Catnach S, Farrant P, Williams R. Diagnostic value of Doppler assessment of the hepatic and portal vessels and ultrasound of the spleen in liver disease. Eur J Gastroenterol Hepatol. 2004;16(2):147-55.
5. Winkfield B, Aubé C, Burtin P, Calès P. Inter-observer and intra-observer variability in hepatology. Eur J Gastroenterol Hepatol. 2003;15(9):959-66.
6. Kardorff R, Gabone RM, Mugashe C, Obiga D, Ramarokoto CE, Mahlert C, et al. *Schistosoma mansoni*-related morbidity on Ukerewe Island, Tanzania: clinical, ultrasonographical and biochemical parameters. Trop Med Int Health. 1997;2(3):230-9.
7. Aquino RT, Chieffi PP, Catunda SM, Araújo MF, Ribeiro MC, Taddeo EF, et al. Hepatitis B and C virus markers among patients with hepatosplenic mansoni schistosomiasis. Rev Inst Med Trop Sao Paulo. 2000;42(6):313-20.
8. Alves A Jr, Fontes DA, Melo VA de, Machado MC, Cruz JF, Santos EA. [Schistosomal portal hypertension: influence of the portal blood flow in serum levels of hepatic enzymes]. Arq Gastroenterol. 2003;40(4):203-8. Portuguese. Comment in: Arq Gastroenterol. 2003;40(4):201-2.
9. Martins RD, Borges DR. Ethanol challenge in non-alcoholic patients with schistosomiasis. J Clin Pathol. 1993;46(3):250-3.
10. Guerra CC, Haddad CM, Matsumoto M, Luzzi JR, da Silva MP, Chacon JP. [Hypersplenism behavior after selective splenorenal anastomosis]. AMB Rev Assoc Med Bras. 1985;31(3-4):65-70. Portuguese.
11. Petroianu A, Oliveira AE, Alberti LR. "Hiperesplenismo" em hipertensão porta por esquistossomose mansônica. Rev Bras Hematol Hemoter. 2004;26(3):195-201.
12. Petroianu A. Pesquisa em Medicina. In: Petroianu A. Ética, moral e deontologia médicas. Rio de Janeiro: Guanabara Koogan; 2000. p.174-8.
13. Petroianu A, Antunes LJ. Imune profiles in hepatosplenic schistosomiasis mansoni after surgical treatments. J Int Med Res. 1998;26(1):43-9.
14. Tanabe M. Haemostatic abnormalities in hepatosplenic schistosomiasis mansoni. Parasitol Int. 2003;52(4):351-9.

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ANEXOS II

Scientific Comments
DOI: 10.5581/1516-8484.20130133

Comment on: the relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis

Autores: Kaiser Junior, Roberto Luiz.

Rev. Bras. Hematol. Hemoter. vol.35 no.6 São José do Rio Preto 2013

http://dx.doi.org/10.5581/1516-8484.20130133

Comment on: the relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis

Número

Scielo e Pubmed

Impact Factor: 0.27

Comment on: Relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis

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Schistosomiasis remains an important public health problem worldwide. It is a parasitic disease endemic in over 70 countries and is estimated that the infection is responsible for more than 200,000 deaths annually[1,2].

For Gryseels et al.[3] inflammatory hepatic schistosomiasis is the main cause of hepatomegaly and severe splenomegaly in children and adolescents. The severity of disease is related to the intensity of the egg infestation. The most severe form of the disease, hepatosplenic schistosomiasis, is an important cause of morbidity and mortality (30% and 10% of those infected, respectively)[4].

Dunn& Kamel[1] showed that Schistosomiasis is one of the most common causes of non-cirrhotic portal hypertension in the world

Schistosomiasis is associated to significant morbidity including anemia, chronic pain, diarrhea, exercise intolerance, malnutrition, bladder cancer, portal hypertension and central nervous system complications[5]. Although most infections occur in residents of endemic areas, it has been clearly documented that brief freshwater exposure is sufficient to establish infection; thus, travelers may also be infected.

From the standpoint of laboratory exams, these patients have leukopenia and significant thrombocytopenia. Sometimes patients have pancytopenia, iron deficiency anemia, leukopenia and thrombocytopenia secondary to great hepatosplenic schistosomiasis[6-8]. We know that both leukopenia and thrombocytopenia are correlated to size of the spleen[9], but there are no studies that directly or precisely correlate splenomegaly and hematologic findings. It is still controversial whether the thrombocytopenia observed in patients with chronic liver disease is more associated to splenomegaly or to very high portal blood pressure.

In the early stages the portal resistance, hypertension is principally presinusoidal. However, due to progressive fibrotic changes in the portal tracts, lobular distortion occurs at the sinusoidal level. This results in increases in resistance to portal venous flow, as evidenced by increased wedged hepatic venous pressure in advanced cases[10]. This may explain possible hematological abnormalities in these patients.

Martins et al.[11] revisited 141 medical records of patients with hepatosplenic schistosomiasis mansoni submitted to the surgical treatment of portal hypertension. The variations in the serum levels of platelets in both the pre- and postoperative periods of these patients were directly correlated to changes in weight and volume of the spleen. Splenomegaly was directly responsible for the variation in the number of platelets. In this study, patients who underwent surgical treatment showed increased serum levels of platelets in the immediate postoperative compared to the preoperative period.

Santos et al.[12] showed that this result refers to the splenic sequestration that occurs in schistosomiasis. The results of this study suggest that ultrasound can be reliably used in the classification of periportal fibrosis using the criteria of Niamey in patients with the advanced form of schistosomiasis. Ultrasound scans in patients with schistosomiasis have been restricted to study the caliber of the portal and splenic veins, and organometric investigations of the portal vein[13].

Some studies reported results relevant to the understanding of the importance of the correlation between splenomegaly and thrombocytopenia in hepatosplenic schistosomiasis. Martins et al.[11], analyzing the serum level of platelets in respect to pre and postoperative weight, tried to correlate this with volume of the spleen in patients with hepatosplenic schistosomiasis with indication for the surgical treatment of portal hypertension. He found that the number of platelets in the immediate postoperative period was inversely correlated with the weight of the spleen removed. Splenomegaly was directly responsible for the variation in the number of platelets.

Thus, the study of Leite et al. is highly relevant since it suggests that the hematological abnormalities are associated with splenomegaly, hypersplenism and hypertension[14]. Further studies are necessary to verify that the platelet count may be a non-invasive tool for portal hypertension.

Conflict-of-interest disclosure:
The author declares no competing financial interest

Submitted: 10/22/2013
Accepted: 10/23/2013

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DOI: 10.5581/1516-8484.20130130

References

1. Dunn MA, Kamel R. Hepatic schistosomiasis. Hepatology. 1981;1(6):653-61.
2. Makdissi FF, Herman P, Machado MA, Pugliese V, D'Albuquerque LA, Saad WA. [Portal vein thrombosis after esophagogastric devascularization and splenectomy in schistosomal portal hypertension patients: what´s the real importance?]. Arq Gastroenterol. 2009;46(1):50-6.Portuguese.
3. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet. 2006;368(9541):1106-18.
4. Ferreira FG, Chin EW, Santos MF, Carvalho DL de, Capua Junior A de. [Portal congestion and thrombosis after esophagogastric devascularization and splenectomy]. Rev Assoc Med Bras. 2005;51(4):233-6. Portuguese.
5. King CH, Dickman K, Tisch DJ. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet. 2005;365(9470):1561-9. Comment in: Lancet. 2005;365(9470):1520-1.
6. Cleva R, Genzini T, Laudanna AA. Hipertensão portal na esquistossomose. Rev Med Univ São Paulo 1996;75:126-9.
7. Cleva R de, Herman P, Saad WA, Pugliese V, Zilberstein B, Rodrigues JJ, et al. Postoperative portal vein thrombosis in patients with hepatosplenic mansonic schistosomiasis: relationship with intraoperative portal pressure and flow. A prospective study. Hepatogastroenterology. 2005;52(65):1529-33.
8. Janini DS, Oliveira IR, Widman A, Ianhez LE, Cerri GG. Aspectos morfológicos e hemodinâmicos do baço em indivíduos normais: estudo por ultrassom Doppler. Radiol Bras. 2003;36(4):213-8.
9. Organização Mundial da Saúde. Departamento de Controle de Doenças Tropicais Negligenciadas. Working to overcome the global impact of neglected tropical diseases [Internet]. Geneva; OMS; 2010. [cited 2013 Jan 21]. Available from: http://whqlibdoc.who.int/hq/2010/WHO_HTM_NTD_2010.2_eng.pdf
10. Silva LC da, Carrilho FJ. Hepatosplenic schistosomiasis. Pathophysiology and treatment. Gastroenterol Clin North Am. 1992;21(1):163-77.
11. Martins RN, Cleva R, Gouveia EM, Ghons NB, Herman P. Correlação entre esplenomegalia e plaquetopenia na forma hepatoesplênica da esquistossomose mansônica. ABCD Arq Bras Cir Dig. 2010;23(4):254-8.
12. Santos GT, Sales DM, Leão AR, Santos JE, Aguiar LA, Brant PE, et al. Reproducibility of ultrasonography in the assessment of periportal fibrosis according to Niamey criteria in patients with schistosomiasis mansoni. Radiol Bras. 2007;40(6):377-81.
13. Machado MM, Rosa AC, Oliveira IR, Cerri GG. Aspectos ultrasonográficos da esquistossomose hepatoesplênica. Radiol Bras. 2002;35(1):41-5.
14. Leite LA, Domingues AL, Lopes EP, Ferreira RC, Filho AA, Fonseca CS, et al. Relationship between splenomegaly and hematologic findings in patients with hepatosplenic schistosomiasis. Rev Bras Hematol Hemoter. 2013;35(5):332-6.

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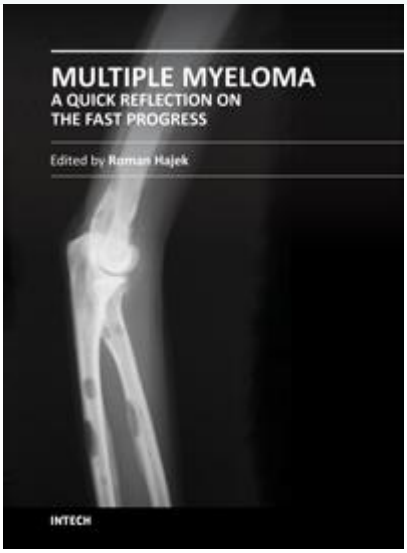
ANEXOS III

Multiple Myeloma - A Quick Reflection on the Fast Progress

Edited by Roman Hajek, ISBN 978-953-51-1083-5, 326 pages, Publisher: InTech, Chapters published April 10, 2013 under CC BY 3.0 license
DOI: 10.5772/56515



Chapter 5

Immunophenotyping in Multiple Myeloma and Others Monoclonal Gammopathies

By Lucie Rihova, Karthick Raja Muthu Raja, Luiz Arthur Calheiros Leite, Pavla Vsianska and Roman Hajek DOI: 10.5772/55938

Immunophenotyping in Multiple Myeloma and Others Monoclonal Gammopathies

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ANEXOS VI

0 011.0.172.000-11	ESTUDO DA COAGULAÇÃO E FIBRINÓLISE EM PORTADORES DE ESQUISTOSSOMOSE HEPATOESPLÊNICA E SUA RELAÇÃO COM APOLIPOPROTEÍNAS PLASMÁTICAS.	Centro de Ciências da Saúde da Universidade Federal de Pernambuco UFPE

Andamento do projeto - - AAE - - 0011.0.172.000-11

Título do Projeto de Pesquisa

ESTUDO DA COAGULAÇÃO E FIBRINÓLISE EM PORTADORES DE ESQUISTOSSOMOSE HEPATOESPLÊNICA E SUA RELAÇÃO COM APOLIPOPROTEÍNAS PLASMÁTICAS.

Situação	Data Inicial no CEP	Data Final no CEP	Data Inicial na CONEP	Data Final na CONEP
Aprovado no CEP	01/02/2011 13:05:04	15/04/2011 14:56:19		

Descrição	Data	Documento	Nº do Doc	Origem
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2 - Recebimento de Protocolo pelo CEP (Check-List)	01/02/2011 13:05:04	Folha de Rosto	0011.0.172.000-11	CEP
4 - Protocolo Aprovado no CEP	15/04/2011 14:56:19	Folha de Rosto	028/11	CEP