Interleukin 17 A, hepatosplenic mansonic schistosomiasis and atherosclerosis

Interleucina 17 A, esquistossomose mansônica forma hepatoesplênica e aterosclerose

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ABSTRACT

Objective: To evaluate serum concentrations of interleukin 17A (IL17A) in mansonic schistosomiasis patients, while the secondary objectives were to detect atherosclerotic disease, and to evaluate serum concentrations of interleukin 22 (IL22). **Methods:** This study included 30 patients with an established diagnosis of hepatosplenic mansonic schistosomiasis and 10 healthy volunteers. Comparative analyses of IL17A and IL22 concentrations were performed on the sera of patients and controls. Atherosclerosis was evaluated through carotid artery intima-media thickness measurement of the first 15 patients enrolled. **Results:** There were no differences in IL17 A concentrations (15.63±0.00pg/mL vs. 15.63±0.00pg/mL; p=1) and in IL22 concentrations (7.81±0.00pg/mL vs. 7.81±0.00pg/mL, p=1) between patients and controls. The overall mean of intima-media thickness was 0.7±0.2mm. **Conclusions:** Serum concentrations of IL17A and IL22 were equal between patients and controls (undetectable or low concentrations). No patients had atheroma.

Keywords: Schistosomiasis mansonic; Atherosclerosis; Inflammation; Immunity

RESUMO

Objetivo: Avaliar as concentrações séricas de interleucina 17A (IL17A) em pacientes com esquistossomose mansônica, enquanto os objetivos secundários foram detectar a doença aterosclerótica e avaliar as concentrações séricas de interleucina 22 (IL22). **Métodos:** Este estudo incluiu 30 pacientes com diagnóstico estabelecido de esquistossomose mansônica em sua forma hepatoesplênica e 10 voluntários saudáveis. Análises comparativas de concentrações de IL17A e IL22 foram realizadas em soros de pacientes e controles. A aterosclerose foi avaliada pela medida do complexo íntima-média dos primeiros 15 pacientes recrutados. **Resultados:** Não houve diferença nas concentrações de IL17A (15,63±0,00pg/mL vs. 15,63±0,00pg/mL; p=1) e nas concentrações IL 2 (7,81±0,00pg/mL vs. 7,81±0,00pg/mL; p=1) entre os pacientes e controles. A média geral da espessura da camada média da íntima foi de 0,7±0,2mm. **Conclusões:** As concentrações séricas de IL17A e IL22 foram iguais entre pacientes e controles (concentrações indetectáveis ou baixa). Nenhum dos pacientes apresentou ateroma.

Descritores: Esquistossomose mansoni; Aterosclerose; Inflamação; Imunidade

INTRODUCTION

Five types of schistosomes parasitize humans: *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma haematobium*, *Schistosoma intercalatum*, and *Schistosoma Mekong*.[1]

It is believed that 700 million people may develop schistosomiasis, and an estimated 200 million people are infected with *S. mansoni* worldwide.[2,3]

The immune system plays an important role in the pathophysiology of schistosomiasis due to its activation of various immune cells and release of different inflammatory mediators.[4,5]

Similarly, atherosclerosis is a disease that involves the immune system and affects millions of people worldwide, causing death or incapacitation. Atherosclerosis is characterized by low chronic inflammation and periods of exacerbation.[6,7] The pathologies of schistosomiasis and atherosclerosis are not yet fully understood.[2,3,6,7]

The main aim of this study was to evaluate the serum concentrations of interleukin 17A (IL17A) in patients with mansonic schistosomiasis, while the secondary objectives were to detect atherosclerotic disease and to evaluate the serum concentrations of interleukin 22 (IL22).

METHODS

This cross-sectional prospective analytical study was conducted between June 2013 and February 2014, and was

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developed in accordance with the ethical principles of clinical research. The study was approved by our institution's clinical research ethics committee.

This study included 30 patients (14 men, 16 women; mean age, 59.87±7.50 years) with an established diagnosis of hepatosplenic mansonic schistosomiasis (sample empirically defined by the researchers) who are monitored in the outpatient clinic of our institution, aged >40 years, who agreed to participate in the study by signing the Free and Informed Consent Form. Patients meeting any of the following criteria were excluded from the study: history of surgery of the bronchial tree, or lung parenchyma; ongoing immunosuppressive therapy; or severe comorbidities, and life expectancy <1 year, coagulopathy, renal failure with creatinine clearance <15ml/min, end stage liver failure, acute disease in the previous month, active rheumatologic disease, autoimmune disease, cirrhosis, hepatitis B or C, alcoholic or nonalcoholic hepatic steatosis, decompensated diabetes mellitus, decompensated heart failure, other heart diseases that cause pulmonary hypertension, or severe chronic obstructive pulmonary disease.

A total of ten healthy volunteers (four men, six women; mean age, 40.93±18.50 years) were selected as controls for interleukin concentrations.

Patients' clinical, social, and demographic characteristics were assessed by anamnesis and physical examination, while their information was collected and stored with the aid of questionnaires.

A 10-mL sample of peripheral blood was collected from each patient and control included in the study and sent to the laboratory for enzyme-linked immunosorbent assay. The lower limits of detection were: IL17A=15.63pg/mL, and IL22=7.81pg/mL.

Comparative analyses of IL 17A and IL 22 concentrations were performed in the sera of patients and controls.

Atherosclerosis was detected by measuring the carotid artery intima-media thickness (CIMT) of the first 15 patients enrolled in the study according to the protocol described below.

CIMT was measured using a Medison X8 ultrasound device with a 7.5-12 MHz linear transducer. The common carotid artery was bilaterally assessed using an automatic software (self-IMT), whereas the bilateral internal and external carotid arteries were studied manually.

field depth was 30 to 40mm, and a gain adjustment with little intraluminal artifacts was detected, while harmonic signals were not used and the cardiac cycle was monitored using image-coupled electrocardiography to verify the end of diastole.[8,9]

A double line representing the three layers of the arterial wall was seen in the posterior wall of the arteries. The first line is the interface between blood and intima (anechoic lumen and echogenic intima), while the second line represents the interface between the middle layer and the adventitia (hypoechoic media and echogenic adventitia).[8-11]

IMT is identified at measurements ≥0.9mm and <1.5mm; however, those ≥1.5mm are indicative of atherosclerotic plaques.[12]

Categorical variables are described as percentages and numerical variables as mean and standard deviation. A descriptive statistical analysis was performed to describe the clinical variables. Shapiro-Wilk test was necessary to determine whether the variables followed a normal distribution. The continuous variables were evaluated using Student's t test. A p value ≤0.05 was considered statistically significant.

RESULTS

No difference between groups was observed in terms of gender, although the patient group had a higher mean age than the control group (59.87±7.50 years vs. 40.93±18.50; p=0.001).

Main patients' clinical characteristics were: benign arrhythmias, 19 patients (63%); hypertension, 17 (56%); smoking, 12 (40%); hepatitis B or C, nine (30%); peripheral vascular insufficiency, nine (30%); family history of CAD, nine (30%); diabetes mellitus, one (3.3%); leukemia, one (3.3%); and non-cardiac surgery, 24 (80%). Informed surgeries included liver segmentectomy, debridement of the lower limb, inguinal hernia repair, hemorrhoidectomy, hysterectomy, perineoplasty, cesarean section, nasal cyst removal, tubal ligation, uterine myomectomy, cataract surgery, cholecystectomy, varicose veins surgery, esophageal variceal ligation, and ovarian excision.

No patient presented with acute coronary syndrome, chronic coronary syndrome, or cerebrovascular accident, or underwent coronary angioplasty (with or without stent) or myocardial coronary artery bypass. None reported dyslipidemia, peripheral artery disease, or kidney failure with a creatinine clearance ≤60mL/min.

All patients were retired, with an average monthly salary of 300 dollars. There were no Caucasians, and all patients were illiterate or semi-illiterate.

There were no differences in the IL17A concentrations (15.63±0.00pg/ml vs 15.63±0.00pg/mL; p=1) and in the IL22 concentrations (7.81±0.00pg/mL vs. 7.81±0.00pg/mL; p=1) between patients and controls.

The overall mean of the intimal medial thickness was 0.7±0.2mm. None of the patients had atheroma.

DISCUSSION

In this study, serum concentrations of IL17A and IL22 were equal between patients and controls (undetectable or low concentrations).

A study conducted in a region with a high prevalence of *S. mansoni* revealed that serum IL 17 concentrations were low or undetectable (15.63pg/mL) in patients with mansonic schistosomiasis and varying degrees of liver fibrosis.[13] In a way, this finding agrees with our results.

No atherosclerosis was observed in the patients of the current study, a finding that is consistent with that of a previous study published in the literature that evaluated patients with hepatosplenic mansonic schistosomiasis who did not undergo portal decompression surgery.[14]

The assessment of carotid atherosclerosis is widely used in the literature, since it is performed in a vascular site in which a non-invasive and early diagnosis can be made even at the subclinical

stage. Moreover, an association between carotid disease with atherosclerosis in the coronary arteries and peripheral arterial disease has been demonstrated.[15,16]

Lipid metabolism has unique traits in schistosomiasis and is partially influenced by the host immune response. Classical dyslipidemia is not common in patients with this parasitosis, although circulating lipoproteins that could be atherogenic were detected in several individuals.[17-19]

While atherosclerosis and schistosomiasis are diseases in which the immune system plays an important role, studies on the patients' immunologic responses revealed several striking differences between the diseases.[4,6]

In mansonic schistosomiasis, the hepatosplenic form of the disease occurs in 8 to 10% of cases. The immunoinflammatory response of the host as well as the presence of juvenile parasites and their eggs during the acute phase of the disease are crucial to the development of tissue fibrosis, which is a hallmark of this disease subtype.[19]

The immune response to egg antigens leads to periportal fibrosis that causes changes in the portal blood flow and contributes to portal hypertension. After a few years, the portosystemic shunt opens, thus allowing the eggs to migrate to the lungs.[20]

The Th2 immune response pathway predominates in this pathology, particularly with the involvement of interleukin 13 (IL13) as a key mediator of granuloma size and hepatic fibrosis.[21] In this context, interleukin 4 and resistant like molecule are also involved in the activation of inflammation and establishment of tissue fibrosis within the liver and lungs.[22]

The acute infection caused by the parasite induces the formation of granulomas through activation of the Th1 pathway, whereas the chronic infection promotes a shift to the Th2 pathway, thus increasing concentrations of certain interleukins such as IL13, IL4, and IL5, and reducing interferon-γ (IFN-γ) concentration.[23,24]

In human atherosclerosis, there is evidence that a patient's immune response via activation of the Th1 pathway contributes to the formation of atheroma and may become predominant. However, controversy persists regarding the role of the Th2 pathway as well as the pro- or anti-atherosclerotic effect of the Th17 pathway.[7,25]

In addition to the importance of lipoproteins in atherosclerosis, various cells and molecules are involved in its pathophysiology, such as Th1-released pro-inflammatory markers, vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1, P-selectin, E-selectin, monocyte chemoattractant protein-1, IL1b, IL6, IL8, IL12, IFN-γ, and macrophages. The dynamic immunoinflammatory balance plays an important role in this disease, and although there is much knowledge available in this area, many aspects remain to be elucidated.[26,27]

In some diseases, the expression of IL 17 has been shown to be associated with polarization of the immune response via Th1 pathway and the interleukins released upon its activation.[13,25,28]

IL 17A, already found in atheroma, exerts a pro-inflammatory effect that increases neutrophil migration to tissues, promotes apoptosis, contributes to endothelial injury, induces the secretion of IL 6, IL 8 and IL 22, and acts in synergy with tumor necrosis factor-α. However, it reduces endothelium VCAM1 concentration and vascular infiltration of T lymphocytes.[28,29]

Several authors have reported that IL 17 deficiency reduces MCP1, IL 1, IL 6, IFN-γ, IL 12 concentrations, and change the polarization of type 1 and 2 macrophages. IL 17A receptor is ubiquitously expressed, which enables it to target a number of cells.[29]

The effects of IL 17A combined with those due to its absence suggest a pro-atherosclerotic effect,[28] although Taleb et al.,[30] in an animal model, demonstrated that this interleukin inhibited the formation of atherosclerotic plaques. However, it is believed that this difference was due to the type of animal feed used, since the pattern differed from those of other studies.

Given that elevated serum concentrations of IL17 are associated with polarization of the immune response via Th1, the fact that an increase of IL17A concentration (hepatosplenic mansonic schistosomiasis in patients with a long disease duration) was not detected in our study, and published data in the literature state that there is a polarized immune response through the Th2 pathway in such patients, we believe that the Th2 pathway was predominant in the patients studied here.

Together with this concept, i.e. a predominance of the Th2 pathway, the absence of atherosclerosis in the patients studied suggests that the polarized immune response induced via Th2 (like that found in mansonic schistosomiasis patients) contributes for protection against atherosclerosis.

In conclusion, the immune response of patients with hepatosplenic mansonic schistosomiasis may attenuate the development of atherosclerosis.

The major limitations of this study are its lack of characterization of cellularity and interleukins of the Th2 pathway, and that the evaluation of atherosclerosis was performed at only one vascular site.

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REFERENCES

1. Ross AG, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, et al. Schistosomiais. N Engl J Med. 2002;346(16):1212-20.
2. Schawartz E. Pulmonary schistosomiasis. Clin Chest Med. 2002; 23(2):433-43.
3. Chitsulo L, Loverde P, Engels D. Schistosomiasis. Nat Rev Microbiol. 2004;2(1):12-3.
4. Gustavson S, Zouain JB, Alves M, Leite F, Goes AM. Modulation of granulomatous hypersentivity against Schistossoma mansoni eggs in mice vaccinated with cultured-derived macrophages loaded with PIII. Parasitol Int. 2002:51(3):259-69.
5. Alves Oliveira LF, Moreno EC, Gazinelli G, Martins Filho AO, Silveira MA, Grazinelli A, et al. Cytokine production associated with periportal fibrosis during chronic schistossomiais mansoni in human. Infect Immun. 2006:74(2):1215-21.
6. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD,

Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation. 2012;125(1):e2-e220. Erratum in: Circulation. 2012;125(22): e1002.

7. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011; 473(7347):317-25.
8. Coll B, Feinstein SB. Carotid intima-media thickness measurements: techniques and clinical relevance. Curr Atheroscler Rep. 2008; 10(5):444-50.
9. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannhein Intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germary, 2004, and brussels, Belgium, 2006. Cerebrovasc Dis. 2007;23(1):75-80.
10. Roman MJ, Naqvi TZ, Gardin JM, Gerhard-Herman M, Jaff M, Mohler E; American Society of Echocardiography; Society of Vascular Medicine and Biology. Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. J Am Soc Echocardiogr. 2006;19(8):943-54.
11. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation. 1986; 74(6):1399-406.
12. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, Ebrahim S, Fatar M, Hernandez Hernandez R, Kownator S, Prati P, Rundek T, Taylor A, Bornstein N, Csiba L, Vicaut E, Woo KS, Zannad F; Advisory Board of the 3rd Watching the Risk Symposium 2004, 13th European Stroke Conference. Mannheim intima-media thickness consensus. Cerebrovasc Dis. 2004;18(4):346-9.
13. de Souza RB, Cardoso LS, Lopes GTV, Almeida MC, Oliveira RR, Alcantara LM, et al. Cytokine and chemokine profile in individuals with diferente degrees of perirpotal fibrosis due to Schistossoma mansoni infection. J Parasitol Res. 2012;2012;394981.
14. Guimarães AV, Brandt CT, Ferraz A. Complexo miointimal das artérias comum e interna em portadores de esquistossomose mansonica hepatoesplenica. Rev Col Bras Cir. 2009:36:292-9.
15. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. 2007;115(4):459-67.
16. O´Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Eng J Med. 1999;340(1):14-22.
17. Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, et al. Effects of infection and inflammation on lipid an lipoprotein metabolism: mechanism and consequences to the host. J Lipid Res. 2004;45(7):1169-96.
18. Dimenstein R, Carvalho VC, Oliveira DN, Gillet MP. Alteration in the levels and lipids composition of plasma lipoprotein (VLDL, LDL and HDL) in Brazilian patients with hepatoesplenic schistosomiasis. Braz J Med Biol Res. 1992;25(11):1091-102.
19. Graham BB, Chabon J, Bandeira A, Espinheira L, Butrous G, Tuder RM. Significant intrapulmonary Schistosoma egg antigens are not present in schistosomiasis-associated pulmonary hypertension. Pulm Circ. 2011;1(4):451-61.
20. Lapa M, Dias B, Jardim CJ, Fernandes CJ, Dourado PM, Figueiredo M, et al. Cardiopulmonary manifestations of hepatosplenic schistosomiasis. Circulation. 2009;119(11):1518-23.
21. Reiman RM, Thompson RW, Feng CG, Hari D, Knight R, Cheewer AW, et al. Interleunkin 5 (IL5) augments the progression of liver fibrosis by regulating IL 13 activity. Infect Immun. 2006;74(3):1471-9.
22. Graham BB, Mentink Kane MM, El Haddad H, Purnel S, Zhang L, Zaiman A, et al. Schistosomiasis induced experimental pulmonar hypertension. Role of interleukin 13 signaling. Am J Pathol. 2010;177(3):1540-61.
23. Mentink-Kane MM, Cheever AW, Thompson RW, Hari DM, Kabatereine NB, Vennervald BJ, et al. IL-13 receptor α2 down-modulates granulomatous inflammation and prolongs host survival in schistosomiasis. Proc Natl Acad Sci USA. 2004;101(2):586-90.
24. Ramalingam TR, Pesce JT, Sheikh F, Cheever AW, Mentink-Kane MM, Wilson MS, et al. Unique functions of the type II interleukin 4 receptor identified in mice lacking the interleukin 13 receptor α1 chain. Nat Immunol. 2008;9(1):25-33.
25. Pober JS. Interleukin 17 and atherosclerosis vascular disease. Arterioscle Thromb Vasc Biol. 2011;31(7):1465-6.
26. Wyss CA, Neidhart M, Altwegg L, Spanaus KS, Yonekawa K, Wischnewsky MB, et al. Cellular actors, Toll-like receptors, and local cytokine profile in acute coronary syndromes. Eur Heart J. 2010;31(12):1457-69.
27. Libby P. Inflammation in atherosclerosis. Nature. 2002;420(6917): 868-74.
28. Hashmi S, Zeng QT. Role of interleukin 17 and interleukin 17 induced cytokines interleukin 6 and interleukin 8 in unstable coronary artery disease. Coron Artery Dis. 2006;17(8):699-706.
29. Yao Z, Fanslow EC Seldin MF, Rousseau AM, Painter SL, Comeau MR, et al. Herpes virus Saimiri encodes a new cytokine, IL 17 which binds to a new cytokine receptor. Immunity. 1995; 3(6):811-21.
30. Taleb S, Romain R, Ramkhelawon C, Uyttenhove C, Pasterkamp G, Herbin O, et al. Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin 17 in atherosclerosis. J Exp Med. 2009;206(10):2067-77.