Rev Soc Bras Med Trop 49(6):781-785, November-December, 2016
doi: 10.1590/0037-8682-0141-2016

Short Communication

There is no evident correlation between interleukin-10 gene polymorphisms and periportal fibrosis regression after specific treatment

***Paula Carolina Valença Silva*[1], *Aline Vieira da Silva*[1], *Taysa Nascimento Silva*[1], *Letícia Moura de Vasconcelos*[1], *Adriana Vieira Gomes*[2],[3], *Maria Rosângela Cunha Duarte Coêlho*[4],[5], *Maria Tereza Cartaxo Muniz*[2],[3],[6] and *Ana Lúcia Coutinho Domingues*[7]**

[1]. Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Vitória de Santo Antão, Pernambuco, Brasil. [2]. Laboratório de Biologia Molecular, Centro de Oncohematologia Pediátrica, Hospital Universitário Oswaldo Cruz, Universidade de Pernambuco, Recife, Pernambuco, Brasil. [3]. Instituto de Ciências Biológicas, Universidade de Pernambuco, Recife, Pernambuco, Brasil. [4]. Setor de Virologia do Laboratório de Imunopatologia Keizo-Asami, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil. [5]. Departamento de Fisiologia e Farmacologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil. [6]. Faculdade de Ciências Médicas, Universidade de Pernambuco, Recife, Pernambuco, Brasil. [7]. Departamento de Medicina Clínica, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil.

Abstract

Introduction: We evaluated the associations between interleukin-10 (IL-10) gene polymorphisms -G1082A/-C819T/-C592A and periportal fibrosis regression after specific treatment for schistosomiasis. **Methods:** This retrospective cohort study involved 125 Brazilian patients infected with *Schistosomiasis mansoni*, who were followed up for 2 years after specific treatment to estimate the probability of periportal fibrosis regression. **Results:** There was no evidence of associations between IL-10 polymorphisms and periportal fibrosis regression after treatment. **Conclusions:** There was no evidence of associations between gene promoter polymorphisms of IL-10 and the regression of periportal fibrosis in this Brazilian population.

Keywords: Interleukin-10. Periportal fibrosis. *Schistosomiasis mansoni*.

Schistosomiasis is a chronic parasitic infection caused by the trematode *Schistosoma*, which affects about 240 million people worldwide[1]. Brazil is notable in the worldwide context of schistosomiasis because about 25 million Brazilians live in areas that present risks of contamination and about 4 to 6 million Brazilians are infected with the *Schistosoma mansoni* species. The Northeastern region of Brazil is particularly notable because of the high endemicity of schistosomiasis, including in the State of Pernambuco, which has the third-greatest prevalence of this disease in the country, as well as the highest number of deaths recorded for this disease[1].

Schistosomiasis causes periportal fibrosis (PPF). The PPF process can result in the development of portal hypertension (PH), which itself leads to splenomegaly and the appearance of varicose veins in the esophagus. The rupture of these esophageal varices with consequent upper gastrointestinal bleeding (UGB) occurs in 12-15% of patients with the hepatosplenic form of the disease[2].

The development of PPF is dependent on various immunogenetic and environmental factors. Sex, age, parasite load, alcohol consumption, exposure frequency, exposure duration, and other factors may affect the development and severity of PPF[3]. On the other hand, specific treatment may also contribute to reducing infection levels and consequently improve the clinical picture of hepatosplenomegaly and PPF[4][5].

In previous studies, it has been observed that the PPF process depends on the action of cytokines, since interleukin-10 (IL-10) has a central role in this regulation[2][6]. The IL-10 secreted by Th2 cells is an important anti-inflammatory, anti-fibrotic cytokine[6]. However, the role of IL-10 in the activation and inhibition of PPF in schistosomiasis is not well understood[7].

The IL-10 gene contains five sequences of nitrogenous bases responsible for protein-coding (exons) on the chromosome that is located in the positions between 1q31 and 1q32[8]. The probable effects on gene transcription and protein production stimulated studies on polymorphisms in the promoter region of this gene, including -G1082A (rs1800870), -C819T IL-10 (rs1800871), and -C592A IL-10 (rs1800872)[8]. The thymine (T) and cytosine (C) alleles at position -819 in the promoter region

Corresponding author: Profa. Ana Lúcia Coutinho Domingues
e-mail: alcdomingues@hotmail.com
Received 15 April 2016
Accepted 8 August 2016

of the IL-10 gene are in linkage disequilibrium with the adenine (A) and C alleles at position -592, respectively[8].

Thus, this study was undertaken to investigate the associations of clinical factors (sex, UGB, specific treatment, and contact with focus) and IL-10 polymorphisms -G1082A/-C819T/-C592A with PPF regression after specific treatment in patients with *S. mansoni*. We specifically sought to investigate these associations in populations of endemic areas of the State of Pernambuco, Northeastern Brazil.

The study was performed retrospectively using a cohort obtained from a previous study of 203 patients who had schistosomiasis and received specific treatment for this disease. Additionally, -G1082A/-C819T/-C592A IL-10 polymorphisms and their respective serum concentrations determined for these patients[9]. The present cohort study included 119 patients infected with *S. mansoni* who were recruited during 2012-2013 and followed-up retrospectively for 2 years after specific treatment for *S. mansoni* to estimate the probability of PPF regression. Sociodemographic and clinical factors were also identified, with an emphasis on specific treatment for *S. mansoni*.

In the previous study[9] with 203 patients, polymorphisms -G1082A/-C819T/-C592A IL-10 in the promoter region of the IL-10 gene were determined alongside their respective serum concentrations. Of the 203 patients in this previous study, the current study excluded 12 who had image pattern A (without PPF) in the first abdominal ultrasound, 66 who did not have a follow-up of 2 years after the first specific treatment for *S. mansoni*, and 6 for whom the genotype frequencies for regions (-C819T/-C592A) of the IL-10 gene were not determined. The final analysis included 119 patients for these regions and 125 patients for the remaining region (-G1082A).

The patients were divided into two groups for each of the polymorphisms that were studied. For polymorphism (-G1082A) IL-10, group 1 (exposed) included 109 patients with polymorphic genotypes (-G1082A) GA/AA IL10, while group 2 (unexposed) included 16 patients with genotype (-G1082A) GG IL-10. For polymorphisms -C819T/-C592A IL-10, group 1 (exposed) included 74 patients with polymorphic genotypes (-819/-592) CT/CA or TT/AA IL-10, while group 2 (unexposed) included 45 patients with genotype (-819/-592) CC/CC IL-10.

All patients were over 18 years of age and treated in the Gastroenterology Clinic of the *Hospital das Clínicas*, Federal University of Pernambuco (HC/UFPE; Recife, Brazil). Each patient was from an area of the State of Pernambuco in which schistosomiasis was endemic.

The study only included patients who had undergone abdominal ultrasound with PPF evaluation following the protocols of Niamey[10] and Cairo[11], both before treatment for schistosomiasis and 2 years after treatment. We excluded patients with a history of other joint hepatic comorbidities, including fatty liver disease, liver cirrhosis, and hepatitis B or C, as well as patients with other clinical forms of schistosomiasis. Patients with hepatitis B or C were excluded based on serology for hepatitis B surface antigen, hepatitis B core antibody, and hepatitis C antibody.

The outcome of interest was PPF regression identified by ultrasonography of the upper abdomen. The main exposures were polymorphisms -G1082A/-C819T/-C592A IL-10 which, in the context of the hypothesis of this study, were investigated for associations with the severity of PPF. The information on these genotypes and their respective serum IL-10 concentrations were obtained in 2012 and 2013[9].

To determine the genotypes (-1082) GA/AA and GG IL-10, (819T/-C592) CT/AC, TT/AA, and CC/CC IL-10, biological samples from all patients were previously submitted to polymerase chain reaction by specific allele, to detect the single base polymorphism in the region promoting the IL-10 gene[8] and thereby define the exposure groups mentioned above. Serum levels of IL-10 were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Biosource; Invitrogen Corporation, Carlsbad, CA, USA), according to the manufacturer's instructions. The results were expressed in pg/ml based on the standard curves (sensitivity <1.0pg/mL).

The other investigated explanatory variables were sex, age, UGB, and contact with contaminated water. Data were collected using a precoded structured questionnaire that was applied to individuals by a single investigator.

The follow-up period was from the moment the specific treatment was first recorded until 2 years afterwards. Relative risks (RRs) and 95% confidence interval (95% CIs) were estimated in bivariate analyses. The significance level was set at 0.05. Epi Info version 3.5.5 (Centers for Disease Control, Atlanta, GA, USA) was used for all statistical analyses. The Kruskal-Wallis test was used to compare the serum levels of IL-10 between groups.

Overall, the average age of patients was 57 years (standard deviation, 13 years). The frequency distributions of the sociodemographic and clinical variables are summarized in **Tables 1** and **2**, comparing the exposure groups for polymorphisms (-C819T/-C592A) and (-G1082A), respectively. The (-C819T/-C592A) polymorphism groups did not differ significantly for any of the investigated factors (**Table 1)**. However, as shown in **Table 2**, women had polymorphism -G1082A more commonly than men (p = 0.044).

There was no significant association between sociodemographic or clinical variables and PPF pattern regression (**Table 3**). Furthermore, there was no significant association between polymorphisms (-C819T/-C592A) IL-10 and the image pattern regression (RR = 0.82; CI = 0.46-1.47, p = 0.666) or between these polymorphisms and the degree of PPF (RR = 0.87, CI = 0.52-1.46, p = 0.761) (**Table 3**). There was also no association between polymorphism (-G1082A) IL-10 and the image pattern regression (RR = 1.71; CI = 0.59-4.92, p = 0.217). In addition, there was no association between polymorphism (-G1082A) IL-10 and the regression degree of PPF (RR = 0.83, CI = 0.41-1.66, p = 0.827). Finally, there was no statistically significant difference in the median levels of IL-10 between the treatment groups (2.3 vs. 3.5ng/mL, respectively, p = 0.9632).

As has already been established in the literature, several environmental factors influence the natural history of PPF, including age, exposure frequency, parasite load, specific treatment,

TABLE 1

Distribution of sociodemographic and clinical variables according to polymorphisms (-C819T/-C592A) *IL-10* in patients with schistosomiasis, State of Pernambuco, Brazil.

	Group 1 (n=74)		Group 2 (n=45)		
Characteristics	**n**	**%**	**n**	**%**	**P-value**
Sex					
male	27	36.5	24	53.3	0.160
female	47	63.5	21	46.7	
Total	74	100.0	45	100.0	
UGB					
yes	41	55.4	25	55.6	0.734
no	33	44.6	20	44.4	
total	74	100.0	45	100.0	
Current contact with the focus					
yes	16	21.6	13	28.9	0.274
no	58	78.4	32	71.1	
total	74	100.0	45	100.0	
Contact with the focus after treatment					
yes	6	8.1	4	8.9	0.429
no	68	91.9	41	91.1	
total	74	100.0	45	100.0	

IL-10: interleukin-10; **n:** number of patients; **UGB:** upper gastrointestinal bleeding; **P-value:** chi-square test.

TABLE 2

Distribution of sociodemographic and clinical variables according to polymorphism (-G1082A) IL-10 in patients with schistosomiasis. State of Pernambuco, Brazil.

	Group 1 (n=109)		Group 2 (n=16)		
Characteristics	**n**	**%**	**n**	**%**	**P-value**
Sex					
Male	42	38.5	11	68.8	0.044
Female	67	68.5	5	32.2	
Total	109	100.0	16	100.0	
UGB					
yes	58	53.2	11	68.8	0.369
no	51	46.8	5	32.2	
total	109	100.0	16	100.0	
Current contact with the focus					
yes	27	24.8	5	31.3	0.804
no	82	75.2	11	68.2	
total	74	100.0	16	100.0	
Contact with the focus after treatment					
yes	9	8.3	2	14.5	0.798
no	100	91.7	14	87.5	
total	109	100.0	16	100.0	

IL-10: interleukin-10; **n:** number of patients; **UGB:** upper gastrointestinal bleeding; **P-value:** chi-square test.

TABLE 3

Bivariate analyses of the associations between sociodemographic variable, clinical variables, polymorphisms (-C819T/-C592A) IL-10, and the regression pattern of periportal fibrosis in patients with schistosomiasis State of Pernambuco, Brazil.

	Regression of periportal fibrosis patterns						
	yes (n=33)		no (n=86)				
Characteristics	**n**	**%**	**n**	**%**	**RR**	**95% CI**	**P-value**
Sex							
male	15	45.5	36	41.9	1.11	[0.62-1.99]	0.882
female	18	54.5	50	58.1	1		
total	33	100.0	86	100.0			
UGB							
yes	18	54.5	48	55.8	0.96	[0.54-1.72]	0.935
no	15	45.4	38	44.2	1		
total	33	100.0	86	100.0			
Treatment UGB							
treated	13	39.3	21	24.4	1.63	[0.92-2.88]	0.163
no treated	20	60.7	65	75.6			
total	33	100.0	86	100.0			
Current contact with the focus							
yes	2	6.1	8	9.3	0.70	[0.20-1.52]	0.439
no	31	93.9	78	90.7	1		
total	33	100.0	86	100.0			
Contact with the focus after treatment							
yes	2	6.1	8	8.9	0.70	[0.20-1.52]	0.439
no	31	93.9	78	91.1	1		
total	33	100.0	86	100.0			
Genotypes (-C819T/-C592A) IL-10							
CT/TT	19	57.6	55	64.0	0.82	[0.46-1.47]	0.660
CC	14	42.4	31	36.0	1		
total	33	100.0	86	100.0			

IL-10: interleukin-10; **n:** number of patients; **RR:** relative risk; **CI:** confidence interval; **UGB:** upper gastrointestinal bleeding; **CT:** heterozygous ; **TT:** Mutant homozygous; **CC:** Wild homozygous. **P-value:** for the relative risk.

and level of education[3][7][12]. Among the investigated patients who were infected with *S. mansoni*, there was no statistically significant difference in any of these factors between the exposure groups. This suggests that there is a need for further cohort studies to assess other risk factors that have not been addressed in the present study, but that might explain the environmental risk factors in the investigated population.

The immune response regulated by the genetics of the host is believed to play a central role in the natural history of PPF. In this study, which involved Brazilian patients infected with *S. mansoni*, no significant association was found between polymorphisms -G1082A/-C819T/-C592A IL-10 and PPF regression. Additionally, there was no difference in serum levels of IL-10 between exposure groups. Recently, Silva et al.[9] evaluated the effects of these polymorphisms on the severity of PPF in 203 patients infected with *S. mansoni* who had different patterns of PPF in the State of Pernambuco. Silva et al. found that only polymorphism (-G1082A) showed risk associations with disease severity in this Brazilian population. The authors concluded that these results may have been influenced by ethnic variations. Silva et al. also found no difference between the mean serum levels of IL-10 between clinical groups. However, in a study of 812 patients with schistosomiasis in an endemic region in Brazil, Grant et al.[13] found a significant association between polymorphism (-T819C) IL-10 and the non-specific immune response, but not with the ovular immune response to *S. mansoni*. Accordingly, these results were not associated with the development of PPF.

Controversial results have been observed in the majority of studies that have compared genotype distributions of polymorphisms of the IL-10 gene promoter with fibrosis severity[12][14][15]. Jin et al.[14] investigated the role of IL-10-592 A/C, IL-10-819 C/T, and IL-10-1082 A/G gene polymorphisms in the development of liver cirrhosis in Asian populations, demonstrating that the IL-10-592 A/C gene polymorphism enhances the risk of liver cirrhosis, and that this gene variant interacts with chronic hepatitis B infection in this population.

Guo et al.[12] analyzed 197 moderate/severe liver fibrosis cases and 426 mild fibrosis controls, as well as 536 cases of liver cirrhosis and 881 non-cirrhosis controls and concluded that the IL10-819 T allele may be a susceptibility factor for liver cirrhosis related to hepatitis C virus in the Japanese population.

On the other hand, Sofian et al.[15] studied 96 patients infected with the hepatitis B virus (HBV) from a reference center for hepatitis in Central Iran, as well as and 31 healthy controls from a blood transfusion organization. The authors sought to evaluate the correlation between HBV and polymorphism (-G1082A) IL-10. They concluded that these genotypes were not significantly different among the controls, patients who had recuperated from HBV, and patients who were carriers of or had chronic HBV. Thus, they found significant corrections with neither the prognosis of infection with HBV, nor consequent hepatic fibrosis. These results could be attributable to the influence of other genes in the progression of HBV, and are not necessarily solely attributable to a direct relationship between the expression of IL-10 and the outcome of infection by HBV.

The results found in the present study do not exclude the possibility that other cytokines and genes influence PPF regression. Therefore, future studies with larger samples are needed to fully analyze these polymorphisms and their respective serum levels of IL-10 serum, to better assess whether there are connections between -G1082A/C819T/-C592A IL-10 polymorphisms, the expression of IL-10, and PPF intensity. If present, such associations may influence PPF regression after specific treatment for schistosomiasis.

In summary, there was no evidence of associations between gene promoter polymorphisms of IL-10 and the regression of PPF in this Brazilian population. Considering the limitations imposed by the sample size of the present study, further studies are recommended to explore the associations between these polymorphisms and the regression of PPF.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the CH/UFPE (CAAE: 03161512.6.0000.5208).

Acknowledgments

The authors thank the leadership of the Gastroenterology Clinic of the *Hospital das Clínicas, Universidade Federal de Pernambuco* (CH-UFPE), for allowing the selection of patients and the use of the Human Molecular Genetics Laboratories of the University of Pernambuco, as well as the use of the Virology Laboratory of Immunopathology Keizo-Asami (LIKA) of the *Universidade Federal de Pernambuco* (UFPE). This study was supported by the UFPE, Brazil.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Financial Support

The *Universidade Federal de Pernambuco* (UFPE), Brazil, provided financial support for molecular biology experiments and dosages of serum TNF-α and IL-10. A.L.C. Domingues is supported by Pró-Reitoria para Assuntos de Pesquisa e Pós-Graduação (PROPESQ) (23076. 019166.2012/63).

REFERENCES

1. World Health Organization (WHO). Programs and projects: schistosomiaisis. A major public health problem. WHO; 2013. Accessed 2014 Dec 4. Available at: http://www.who.int/schistosomiaisis/en/
2. Andrade ZA. Schistosomiasis and liver fibrosis. Parasite Immunol 2009*;* 31:656-663.
3. Silva PCV, Leal TV, Domingues ALC. Treatment and education reduce the severity of schistosomiasis periportal fibrosis. Rev Soc Bras Med Trop 2013; 46:472-477.
4. Voieta I, Queiroz LC, Andrade LM, Silva LCS, Fontes VF, Barbosa Jr A, et al. Imaging techniques and histology in the evaluation of liver fibrosis in hepatoesplenic schistosomiasis mansoni in Brazil: a comparative study. Mem Inst Oswaldo Cruz 2010; 105:414-421.
5. Domingues ALC. Diagnóstico por imagem. *In:* Carvalho OS, Coelho PMZ, Lenzi HL, organizadores. *Schistosoma mansoni* e esquistossomose: uma visão multidisciplinar. Rio de Janeiro: Fiocruz; 2008. p. 927-961.
6. Peter J, Frey O, Stallmach A, Bruns T. Attenuated antigen-specific cell responses in cirrhosis are accompanied by elevated serum interleukin-10 levels and down-regulation of HLA-DR on monocytes. BMC Gastroenterol 2013;13:37.
7. Arnaud V, Li J, Wang Y, Fu X, Mengzhi S, Luo X, et al. Regulatory Role of interleukin-10 and interferon-γ in severe hepatic central and peripheral fibrosis in humans Infected with *Schistosoma japonicum*. J Infect Dis 2008; 198:418-426.
8. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997; 24:1-8.
9. Silva PCV, Gomes AV, de Souza TK, Coêlho MR, Cahu GG, Muniz MT, et al. Association of SNP (-G1082A) IL-10 with increase in severity of periportal fibrosis in schistosomiasis, in the Northeast of Brazil. Genet Test Mol Biomarkers 2014; 18:646-652.
10. Richter J, Domingues AL, Barata CH, Prata AR, Lambertucci JR. Report of the second satellite symposium on ultrasound in schistosomiasis. Mem Inst Oswaldo Cruz 2001; 96 (suppl):151-156.
11. Cairo Working Group; Jenkins JM, Hatz C, editors. The use of diagnostic ultrasound in schistosomiasis – attempts at standardization of methodology. Acta Trop 1992; 51:45-63.
12. Guo PF, Jin J, Sun X. Influence of IL10 gene polymorphisms on the severity of liver fibrosis and susceptibility to liver cirrhosis in HBV/HCV-infected patients. Infect Genet Evol 2015; 30:89-95.
13. Grant AV, Araujo MI, Ponte EV, Oliveira RR, Cruz AA, Barnes KC, et al. Polymorphisms in IL10 are associated with total Immunoglobulin E levels and *Schistosoma mansoni* infection intensity in a Brazilian population**.** Genes Immun 2011; 12:46-50.
14. Jin XY, Wang YQ, Yan T, Wang J, Qing S, Ding N, et al. Interleukin-10 gene promoter polymorphism and susceptibility to liver cirrhosis. Hepatogastroenterol 2014; 61:442-446.
15. Sofian M, Kalantar E, Aghakhani A, Hosseini S, Banifaz M, Eslamifar A, et al. No correlation between interleukin-10 gene promoter polymorphisms and hepatitis B virus infection outcome. Hepat Mon 2013; 13:e8803. doi: 10.5812/hepatmon.8803.