v. 3, n. 42, 2023

International Journal of Health Science

EVALUATION OF THE PROMOTER POLYMORPHISM (-G1082A) OF THE INTERLEUKIN 10 (IL-10) GENE IN THE SEVERITY OF UPPER GI HEMORRHAGE

Ana Risoflora Alves de Azevedo
``Centro Acadêmico de Vitória``,
``Universidade Federal de Pernambuco``,
Vitória de Santo Antão, Pernambuco, Brazil.

Maria Clara Silva Bezerra
``Centro Acadêmico de Vitória``,
``Universidade Federal de Pernambuco``,
Vitória de Santo Antão, Pernambuco, Brazil.

Aline de Melo Silva
``Centro Acadêmico de Vitória``,
``Universidade Federal de Pernambuco``,
Vitória de Santo Antão, Pernambuco, Brazil.

Taynan da Silva Constantino
``Centro Acadêmico de Vitória``,
``Universidade Federal de Pernambuco``,
Vitória de Santo Antão, Pernambuco, Brazil.

Lucas Emanuel de Vasconcelos Cândido
``Centro Acadêmico de Vitória``,
``Universidade Federal de Pernambuco``,
Vitória de Santo Antão, Pernambuco, Brazil.

Paula Carolina Valença Silva
``Centro Acadêmico de Vitória``,
``Universidade Federal de Pernambuco``,
Vitória de Santo Antão, Pernambuco, Brazil.

Abstract: **Introduction:** Genotypic variants of the *IL-10 gene* may participate in the molecular mechanisms of Periportal Fibrosis (PPF) and, possibly, of Upper Digestive Hemorrhage (HDA) in schistosomal patients. **Method:** This is a cross-sectional study involving 124 individuals infected with *Schistosoma mansoni,* after specific treatment, to verify the association between the genetic polymorphism (-G1082A) *IL-10* and serum concentrations of *IL-10* with HDA. **Results:** There was no evidence of association between the (-G1082A) *IL-10 polymorphism* and its serum concentrations with HDA. **Conclusions:** The (-G1082A) *IL-10 polymorphism and serum IL-10* levels were not associated with UDH in this population.
Keywords: Schistosomiasis mansoni. Interleukin-10. periportal fibrosis. Genetic polymorphism. High digestive bleeding.

Brazil is the main source of transmission of Schistosomiasis Mansoni (MS) in Latin America, with about 1.5 million Brazilians affected by the disease, and it has a high prevalence in the northeast region of the country, especially in the state of Pernambuco, in that the frequency of cases among inhabitants of the Zona da Mata ranges from 10 to 50%. [1-2] Therefore, MS is a neglected parasitic disease that represents a major challenge for Brazilian public health. [1.3]

About 6 weeks after infection with *Schistosoma mansoni,* parasite eggs are deposited in host tissues (liver and intestine or bladder). [4] In response to the antigens secreted by the eggs, the cellular immune response mediated by Th2-CD4 lymphocytes induces the formation of granulomas around the parasite eggs. [4] However, when the host's immune response becomes persistent, fibrosis forms. [4]

Periportal fibrosis (PPF) represents an important indicator of *S. mansoni infection.* [5] Schistosomal FPP is characterized by collagen accumulation in the Hepatic Portal System (PHS) and Space of Disse, which results in fibrosis and sinusoidal capillarization. [5] Fibrosis induces an increase in intrahepatic vascular resistance and blood flow in the HPS and leads to progressive occlusion of the portal vein and the development of portal hypertension. [5] To compensate for these changes, collateral vessels, which manifest clinically as gastroesophageal varices, develop to decompress the HPS. [5] When they rupture, gastroesophageal varices cause Upper Digestive Hemorrhage (UGI). [5]

Among the immunogenetic markers involved in the process of fibrogenesis, Interleukin-10 (*IL-10*) stands out, an anti-inflammatory, anti-immune and anti-fibrotic cytokine that regulates the inflammatory response and the formation of hepatic fibrosis. [2] Thus, it is possible that the genotypic variants of the *IL-10 gene* may participate in the molecular mechanisms involved in PPF and, possibly, in UDH, in individuals affected by MS. [two]

IL-10 gene contains 5 exons located between bands 1q31 and 1q32 of chromosome 1 and several Single Nucleotide Polymorphisms (SNPs) have been described in its promoter region, which may be responsible for the wide variation in the levels of this cytokine between individuals. [2,6-7] Among the polymorphisms described in this region, the polymorphism (-G1082A) is included (rs1800870), which consists of replacing a nucleotide Guanine (G) with Adenine (A) at position -1082, and was associated with PPF in individuals with MS. [2,6] The GG genotype is related to a high production of *IL-10* while GA and AA are related to an intermediate and low production of the cytokine, respectively. [2.6]

Thus, the present study evaluated the association between the polymorphism (–G1082A) of the *IL-10 gene* and the severity

of upper gastrointestinal bleeding in schistosomal individuals in Pernambuco, Northeastern Brazil.

This is a cross-sectional study, carried out between September 2021 and May 2022, with 124 individuals infected with *S. mansoni,* divided into two groups: Group 1 - 68 individuals with the hepatosplenic form (HES) of the disease, with Advanced FPP (Standard E or F by Niamey classification) and HDA; Group 2 - 56 individuals with the hepatointestinal form (HEI) of the disease, with mild PPF (Pattern C by the Niamey classification) or without fibrosis (Pattern A by the Niamey classification) and without UDH. All participants were 18 years of age or older, were treated at the Gastroenterology Outpatient Clinic of ``Hospital das Clínicas``, ``Universidade Federal de Pernambuco`` (HC/UFPE) and came from endemic areas for Schistosomiasis in the state of Pernambuco, Brazil.

The HGS pattern of the participants was stratified using the Niamey Classification. [8] Subjects were included, confirmed by ultrasound of the abdomen, with EHE, with advanced HGS (Pattern E or F by the Niamey Classification), with UDH and splenomegaly or history of previous splenomegaly; and HIE, with mild PPF (Pattern C by the Niamey Classification) or without fibrosis (Pattern A by the Niamey Classification) and without UDH.

Individuals with other liver diseases, such as liver cirrhosis, steatosis, Hepatitis B or C and alcoholic disease, excluded by clinical history, USG of the abdomen and specific laboratory tests (HBsAg, anti-HBc, anti-HBs and anti-HCV); Patients submitted to blood transfusion within a period of less than three months; Other clinical forms of schistosomiasis already diagnosed, such as pulmonary vascular disorders, pseudoneoplastic forms, schistosomal and spinal cord nephropathy. Individuals with a moderate HGS pattern (Pattern D according to the Niamey Classification) were also excluded. Figure 1 shows the participants' eligibility flowchart.

This study was conducted within the standards required by the Declaration of Helsinki and approved by the Research Ethics Committee (CEP) of the Health Sciences Center of ``Universidade Federal de Pernambuco`` (CCS-UFPE), under Protocol 113.199 and CAAE 03161512.6.0000.5208. All participants signed the Free and Informed Consent Term (TCLE).

Peripheral blood samples from participants were collected in tubes containing EDTA (BD Vacutainer, Preanalytical Solutions Franklyin Lakes, NJ) and were transported to the laboratory on ice. Genomic DNA was extracted from peripheral blood leukocytes by the phenol-chloroform method of molecular analysis, adapted from Sambrook and Russel (2001). [9]

The polymorphism (–G1082A) of the *IL-10 gene* was determined by Allele-Specific Polymerase Chain Reaction. Serum *IL-10* levels were measured by commercial enzyme-linked immunoassay (ELISA) (Biosource; Invitrogen Corporation, Carlsbad, CA) according to the manufacturer's instructions. Results were expressed in *pg/mL,* based on the curve pattern (sensitivity <1.7 pg/mL). The average 3 *pg/ml* was used as the cutoff point between the comparison groups, with 36 individuals assessed at this stage.

Epi-Info software, version 3.5.5 (CDC, Atlanta, GA) was used for data analysis. Crude Prevalence Ratio (PR) and 95% confidence intervals (95% CI) were calculated using univariate analyzes to verify the association between genotypic frequencies of the IL-10 (-G1082A) polymorphism with *HDA*. The association was considered significant when $p< 0.05$.

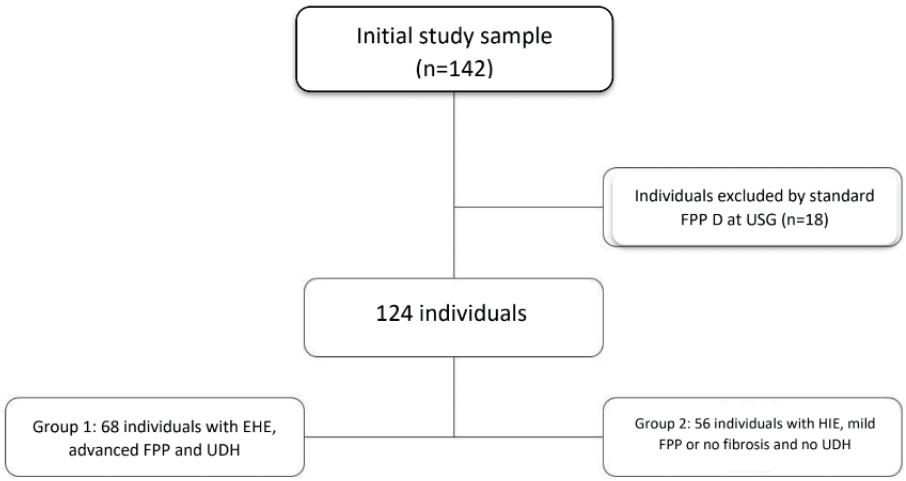

Figure 1. Participant eligibility flowchart.

	HDA	(n=124)			
GENOTYPES	**YES (68)**	**NO (56)**	**RP**	**CI 95%**	**P-VALUE**
	N (%)	**N (%)**			
GA/YY	35 (51.47)	36 (63.64)	0.795	[0.577-1.085]	0.210
GG	33 (48.53)	20 (36.36)			
TOTAL	68 (100)	56 (100)			

Table 1 - Association analysis between polymorphism (-G1082A) and UDH in patients with schistosomiasis mansoni, Pernambuco, Brazil, 2022.

	HDA				
SERUM *IL-10 LEVELS* (AVERAGE)*	**YES N (%)**	**NO N (%)**	**RP**	**CI 95%**	**P-VALUE**
>3pg/ml	19 (76)	6 (54.55)	1,393	[0.778-2.495]	0.371
<3pgml	6 (24)	5 (45.45)			
TOTAL	25 (100)	(100)			

*36 participants were assessed for serum *IL-10 levels* by commercially available enzyme-linked immunosorbent assay (ELISA).

Table 2 - Association analysis between mean serum levels *of IL-10* and HDA in patients with schistosomiasis mansoni, Pernambuco, Brazil, 2023.

	GENOTYPES				
IL-10 SERUM LEVELS	**1 (GA/YY) N (%)**	**2 (GG) N (%)**	**RP**	**CI 95%**	**P-VALUE**
>3pg/ml	17 (73.91)	7 (63.64)	1,180	[0.669-2.082]	0.831
<3pg/ml	06 (26.09)	4 (36.36)			
TOTAL*	23 (100)	11 (100)			

*36 individuals were analyzed for serum IL-10 levels, two individuals did not amplify for PCR reactions.

IL-10 levels and polymorphism (-G1082A).

There was no evidence of a statistically significant association between the polymorphism (–G1082A) and UDH between clinical groups (PR = 0.795, 95% CI = [0.577-1.085] and p= 0.210) (Table 1).

Silva et al (2014) evaluated the AA, AG and GG biallelic polymorphisms in 203 individuals infected with *S. mansoni* in Pernambuco and concluded that there was a risk association between the (-1082) AA *IL-10* and AA + AG genotypes with the advanced pattern of FPP when compared to the (-1082) GG *IL - 10 genotype*.

In another study, which evaluated 119 schistosomal individuals in Pernambuco, no significant association was found between the polymorphism (-G1082A) and the regression of the HGS pattern. [7] The authors concluded that further studies are needed, with larger samples, to evaluate *IL-10* gene polymorphisms and serum *IL-10 levels* to better analyze whether there are possible associations between the polymorphisms (-G1082A/-C819T/-C592A) *IL-10, IL-10* expression and periportal fibrosis intensity. [7]

Table 2 showed no evidence of a statistically significant association between mean serum levels of *IL-10* and HDA between clinical groups (PR = 1.393, 95% CI = [0.778-2.495] and p=0.371).

Mutengo *et al* (2018) measured serum levels of *IL-2, IL-4, IL-6, IL-10, IL-13, IL-17A,* Tumor Necrosis Factor-alpha (TNF- γ) and Interferon-gamma (IFN- *y*) from 244 residents of a hyperendemic region of western Zambia and found evidence that high levels of *IL-6, IL-10* and TNF- α may have a protective effect against Liver Fibrosis (FH) in schistosomal individuals. The study showed the regulatory role of *IL-10,* possibly in synergy with *IL-6,* in regulating the immune response and preventing the development of FH. [10] Low levels of *IL-10* and high levels of *IL-13* have been associated with moderate to severe FH. [10]

Accordingly, Booth *et al* (2004) conducted a cohort in Uganda, in which they analyzed the cellular and demographic immune response of 199 individuals aged between 6 and 50 years and with periportal fibrosis detectable by ultrasound, and also found an association of low levels of *IL- 10,* combined with high levels of *IL-5* and *IL-13,* as an important risk factor for the development of fibrosis. The study demonstrated that the FPP is influenced by the profile of cytokines and chemokines, which vary according to the age and gender of the individuals. [11]

Another study [12,] with fishermen from an endemic area for *S. japonicum* in China, found an association between low levels of *IL-10* and a high risk of developing advanced or severe PPF. Their findings also associated *IL-10* with splenomegaly and hypersplenism, regardless of HGS, indicating a possible direct relationship between them. [12] In view of this, the study suggested that this interleukin appears to have a key role in regulating the pathogenesis of the disease. [12]

In contrast, in the study by Jesus *et al* (2004) serum levels of *IL-5, IL-10, IL-13,* TNF- α, IFN-α and Transforming Growth Factor-beta (TGF- β) were measured in supernatants of peripheral blood mononuclear cells stimulated with soluble egg antigen from 94 patients infected with *S. mansoni* and found elevated levels of *IL-10* in patients with a higher degree of liver fibrosis (Grade III) compared to patients who had a lower degree of fibrosis (Grade I and II).

Therefore, the role of *IL-10* in the severity of PPF in schistosomal individuals is still unclear.

Finally, Table 3 showed no evidence of a statistically significant association between mean serum levels of *IL-10* and polymorphism (–G1082A) between clinical groups (PR = 1.180, 95% CI = [0.669-2.082] and p= 0.831).

Silva et al (2014) evaluated 119 individuals with EHE and advanced HGS and 74 individuals

with HHE and little to moderate HGS or no fibrosis, and found no significant association between mean levels of IL-10 and *HGS*. The study also showed no association between *IL-10* (-G1082A) polymorphism and serum levels of this cytokine. [2] The authors suggest that the (-1082) AA *IL-10* and AA+GA *IL-10 genotypes* may be risk factors for severe PPF and recommend further studies with a larger sample to assess the possible direct relationship between the expression of this cytokine with the outcome of the disease. [two]

IL-10 serum levels, and associated the (-1082) GG genotype with high *IL-10 production* [6,14,] the (-1082) GA genotype with an intermediate production, and the (-1082) AA genotype with a low interleukin production. [6.15]

The results suggest that the (-G1082A) *IL-10 polymorphism and serum IL-10* levels were not associated with UDH in schistosomal individuals. Considering the limitations imposed by the sampling of the present study, it is suggested that additional research be carried out, with a larger sample, to better evaluate the impact of this polymorphism in the prediction of schistosomal UDH.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

This study was funded by ``Universidade Federal de Pernambuco`` (UFPE), Brazil, with financial support from Grupo de Pesquisa/ PROPESQ, 2014, under process number 038882/2014-10.

REFERENCES

1. CALASANS, Taíssa Alice Soledade; SOUZA, Geza Thais Rangel; MELO, Claudia Moura; MADI, Rubens Riscala; JERALDO, Verónica de Lourdes Sierpe. Socioenvironmental factors associated with Schistosoma mansoni infection and intermediate hosts in an urban area of northeastern Brazil. **Plos One**, [S.L.], v. 13, n. 5, p. 1-14, 2 maio 2018.

2. SILVA, Paula Carolina Valença; GOMES, Adriana Vieira; SOUZA, Tayllanne Karina Gomes de; COêLHO, Maria Rosângela Cunha Duarte; CAHU, Georgea Gertrudes de Oliveira Mende; MUNIZ, Maria Tereza Cartaxo; DOMINGUES, Ana Lúcia Coutinho. Association of SNP (-G1082A) IL-10 with Increase in Severity of Periportal Fibrosis in Schistosomiasis, in the Northeast of Brazil. **Genetic Testing And Molecular Biomarkers**, [S.L.], v. 18, n. 9, p. 646-652, set. 2014.

3. MELO, Andrea Gomes Santana de; IRMÃO, José Jenivaldo de Melo; JERALDO, Verónica de Lourdes Sierpe; MELO, Cláudia Moura. Schistosomiasis mansoni in families of fishing workers of endemic area of Alagoas. **Escola Anna Nery**, [S.L.], v. 23, n. 1, p. 1-10, 10 dez. 2018.

4. MEWAMBA, Estelle *et al*. The Genetics of Human Schistosomiasis Infection Intensity and Liver Disease: a review. **Frontiers In Immunology**, [s.l.], v. 12, p. 1-15, 15 fev. 2021.

5. GUNDA, Daniel *et al*. Morbidity and Mortality Due to Schistosoma mansoni Related Periportal Fibrosis: could early diagnosis of varices improve the outcome following available treatment modalities in sub saharan africa? a scoping review. **Tropical Medicine And Infectious Disease**, [s.l.], v. 5, n. 1, p. 20-32, 3 fev. 2020.

6. TURNER, D. M.; WILLIAMS, D. M.; SANKARAN, D.; LAZARUS, M.; SINNOTT, P. J.; HUTCHINSON, I. V.. AN INVESTIGATION OF POLYMORPHISM IN THE INTERLEUKIN-10 GENE PROMOTER. **European Journal Of Immunogenetics**, [S.L.], v. 24, n. 1, p. 1-8, fev. 1997.

7. SILVA, P.C.V. *et al*. There is no evident correlation between interleukin-10 gene polymorphisms and periportal fibrosis regression after specific treatment. **Revista da Sociedade Brasileira de Medicina Tropical**, [s.l.], v. 49, n. 6, p. 781-785, nov./ dez. 2016.

8. RICHTER, J. et al.. Report of the Second Satellite Symposium on Ultrasound in Schistosomiasis. **Memórias do Instituto Oswaldo Cruz**, [s.l.], v. 96, 2001.

9. SAMBROOK, J; RUSSELL, D. W.. MOLECULAR CLONING: A Laboratory Manual. 1ª edição. New York: Cold Spring Habor, 2001.

10. MUTENGO, Mable M.; MDULUZA, Takafira; KELLY, Paul; MWANSA, James C. L.; KWENDA, Geoffrey; MUSONDA, Patrick; CHIPETA, James. Low IL-6, IL-10, and TNF-α and High IL-13 Cytokine Levels Are Associated with Severe Hepatic Fibrosis in Schistosoma mansoni Chronically Exposed Individuals. **Journal Of Parasitology Research**, [S.L.], v. 2018, p. 1-8, 2018.

11. BOOTH, Mark *et al*. Periportal fibrosis in human Schistosoma mansoni infection is associated with low IL-10, low IFN-gamma, high TNF-alpha, or low RANTES, depending on age and gender. **The Journal of Immunology**, [s.l.], v. 172, n.2, p. 1295-1303, 15 jan. 2004.

12. ARNAUD, Violaine; LI, Jun; WANG, Yuanyuan; FU, Xiao; MENGZHI, Shi; LUO, Xinsong; HOU, Xunya; DESSEIN, Helia; JIE, Zhou; XIN-LING, Yu. Regulatory Role of Interleukin–10 and Interferon-γ in Severe Hepatic Central and Peripheral Fibrosis in Humans Infected with Schistosoma japonicum. **The Journal Of Infectious Diseases**, [S.L.], v. 198, n. 3, p. 418-426, ago. 2008.

13. JESUS, Amelia *et al*. Association of Type 2 Cytokines with Hepatic Fibrosis in Human Schistosoma mansoni Infection**. Infection and Immunity**, [s.l.], v. 72, n. 6, p. 3391-3397, Jun. 2004.

14. Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Hohler T. Differential regulation of interleukin-10 production by genetic and environmental factors – a twin study. **Genes Immun**, [s.l.], v.3 p. 407-413, 2002.

15. Świątek, B. J.. Is interleukin-10 gene polymorphism a predictive marker in HCV infection?. **Cytokine & Growth Factor Reviews**, [s.l.], v.23, n.1-2, p. 47–59, 2012.