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EVALUATION OF THE PROMOTER POLYMORPHISM (-C819T) OF THE GENE INTERLEUKIN-10 (IL-10) IN THE SEVERITY OF UPPER GASTROINTESTINAL BLEEDING

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Abstract: Introduction: Upper gastrointestinal bleeding (HDA) is a serious consequence of schistosomiasis mansoni, resulting from the inflammatory process mediated by IL-10. Method: This is a cross-sectional study involving 123 individuals infected with Schistosoma mansoni, after specific treatment, to verify the association between the genetic polymorphism (-C819T) IL-10 and its serum concentrations with HDA. Results: There was no evidence of an association between the (-C819T) IL-10 polymorphism and its serum concentrations with HDA. Conclusions: The IL-10 (-C819T) polymorphism and levels of *IL-10*were not associated with HDA in this population.
Keywords: Schistosomiasis mansoni, Interleukin-10, Periportal Fibrosis, Genetic polymorphism.

INTRODUCTION

Schistosomiasis mansoni (EM) affects approximately 240 million people in 78 countries, and approximately 700 million individuals live in endemic areas.[1] Pernambuco, among the Brazilian states, has a high endemicity for EM.[2,3] The chronic form of schistosomiasis mansoni is present in about 22 million people, and 42% of those infected develop periportal fibrosis (FPP).[4]

Among the immunogenetic factors involved in the inflammatory response in the chronic phase of EM, the cytokine Interleukin-10 (IL-10) stands out, an anti-inflammatory cytokine secreted by Th2 lymphocytes in which its serum levels are associated with FPP. IL-10 transcription is determined by five polymorphisms located on chromosome 1[5], among which the IL-10 polymorphism (rs1800871) stands out, which is located at position -819 of chromosome 1 and consists of the exchange of the nitrogenous base Cytosine for Thymine.[5].

Studies on the impact of IL-10 on the

pathophysiology of MS are controversial. Low serum levels of IL-10 were associated with the severe form of FPP in schistosomiasis[6]. While there are reports that the development of FPP has been related to elevated IL-10 levels.[7,8] Additionally, the (-C819T) IL-10 polymorphism has not been associated with the advanced pattern of FPP in individuals infected with S. Mansoni in Pernambuco.[9] More is needed studies to elucidate the impact of polymorphism (-C819T) of the IL-10 gene in the severe form of EM.

This work investigated the influence of the polymorphism in the IL-10 promoter gene (-C819T) and its serum concentrations on the severity of HDA in individuals infected with S.mansoni, in Pernambuco.

This is a retrospective analytical cross-sectional study carried out between January 2021 and July 2022, with 123 individuals infected with S.mansoni, who were divided into 2 groups: Group 1 - 68 individuals with the hepatosplenic (HE) form of the disease with advanced periportal fibrosis (Standard E or F), with HDA. Group 2 - composed of 55 individuals with the hepatointestinal form (HI), with mild FPP (Pattern C, or without fibrosis (Pattern A) and without HDA. All aged 18 years or older, from endemic areas for Schistosomiasis, with an epidemiological history and specific treatment for EM, attended at the Gastroenterology Outpatient Clinic of the Hospital das Clínicas of the Universidade Federal de Pernambuco (HC/UFPE) in the year 2013. Initially, 142 individuals infected with S.mansoni were enrolled. Of these, 19 individuals were excluded who had standard D FPP on abdominal ultrasonography (US) (Figure 1).

The Niamey Classification was used to stratify the pattern of FPP. Thus, individuals who had USG of the abdomen confirmed for advanced FPP (Pattern E or F) with HDA and splenomegaly or history of previous splenectomy; and in the hepatointestinal (EHI) form, mild FPP (Pattern C) or without fibrosis (Pattern A) and without HDA were included in the study.10

Individuals with other liver diseases such as liver cirrhosis, steatosis, hepatitis B or C, and alcoholic disease that were ruled out by clinical history, abdominal US, and specific laboratory tests (HBsAg, anti HBc, anti HBs, and anti HCV) were excluded; other clinical forms of schistosomiasis already diagnosed, such as pulmonary vascular disorders, pseudoneoplastic forms, schistosomal and medullary nephropathy, and individuals with FPP Standard D (moderate by Niamey Classification).

The determination of genotypes (819T/-C592) CT, TT and CC IL-10, was performed in 2013, through the biological samples of all patients. They were previously submitted to polymerase chain reaction to detect the single base polymorphism in the promoter region of the IL-10 gene.11 The serum IL-10 dosages were performed in 2013 by commercial enzyme-linked immunosorbent assay (ELISA) (Biosource;Invitrogen Corporation, Carlsbad, CA), according to the manufacturer's instructions. Results were expressed in pg/mL, based on the standard curve (sensitivity <1.7pg/mL). The mean 3pg/mL was used as the cutoff point between comparison groups,with 37 subjects evaluated 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 by univariate analysis to verify the association between genotype frequencies of the IL-10 (-C819T) polymorphism with HDA. The association was considered significant when $p< 0.05$.

The study was conducted according to the Helsinki Declaration and approved by the

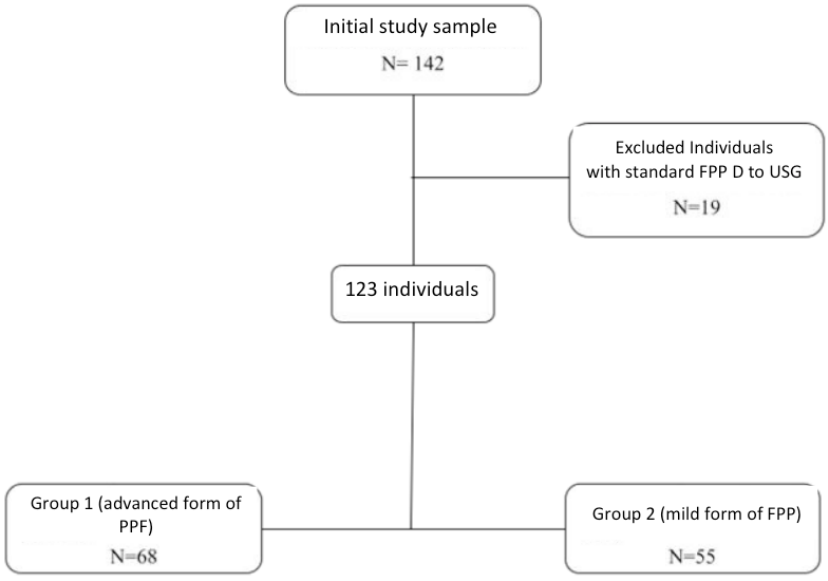

Figure 1- Participant eligibility flowchart.

HDA							
		Yes		No			
Serum dosage* IL-10	**n**	**%**	**n**	**%**	**RP**	**IC 95%**	**p-val**
> 3pg/ml	**21**	**84%**	**7**	**58.3%**	**1.68**	**[0.78-3.61]**	**0.092**
< 3pg/ml	**4**	**16%**	**5**	**41.7%**			
Total	**25**	**100**	**12**	**100**			
Polymorphism -C819T	**n**	**%**	**n**	**%**	**RP**	**CI 95%**	**p-val**
TT/CT	**35**	**51.4%**	**35**	**63.6%**	**0.803**	**[0.29-1.25]**	**0.120**
CC	**33**	**48.6%**	**20**	**36.4%**			
Total	**68**	**100%**	**55**	**100%**			

37 patients were assessed for serum IL-10

Table 1- Analysis of the association between polymorphism (-C819T) IL-10, serum levels of IL-10 and HDA, Pernambuco, 2022.

Polymorphism (-C819T) IL-10							
		Yes		No			
Serum concentration*	**n**	**%**	**n**	**%**	**RP**	**CI 95%**	**p-val**
> 3pg/ml	**17**	**77.2%**	**9**	**75%**	**1.04**	**[0.57-1.91]**	**0.598**
< 3pg/ml	**5**	**22.8%**	**3**	**25%**			
Total	**22**	**100%**	**12**	**100%**			

*37 patients were evaluated for serum IL-10 dosage, of these only 34 samples amplified for the polymorphism (-C919T).

Table 2- Univariate analysis of association between polymorphism (-C819T) and IL-10 levels, Pernambuco, 2022.

Ethics and Research Committee of the Health Sciences Center, UFPE, under protocol113.199 and CAAE 03161512.6.0000.5208.

The mean age of participants was 56 years (DP 13 years). There was no evidence of statistically significant association between gender (CI= [0.877-1.616], p-value= 0.353) and contact with contaminated water (IC= [0.723-1.874], p-value= 0.796) and HDA.

There was no evidence of a statistically significant association between mean serum IL-10 levels and HDA (RP = 1.68, 95% IC = [0.78-3.61]; p=0.092). There was no evidence of a statistically significant association between polymorphisms in the IL-10 promoter region (-C819T) and HDA (RP = 0.803, 95% CI = [0.29-1.25] and p= 0.120) (Table 1).

There was no evidence of a statistically significant association between the C819T-polymorphism and serum IL-10 concentrations (RP = 1.04, 95% IC = [0.57-1.91]; p=0.598)(Table 2).

The HDA is a consequence of chronic cases of schistosomiasis. It is estimated that HDA occurs in up to 80% of people with FPP, with recurrent episodes being frequent, with a mortality rate for each bleeding episode of up to 30%. Not all patients with advanced fibrosis develop esophageal varices, and not all patients with esophageal varices will have HDA, and HDA frequencies in Brazil are lower than in Africa.[4]

Immune response regulated by host genetics plays a central role in the natural history of FPP and possibly in the development of HDA. In this study, which involved Brazilian patients infected with S. mansoni, no significant association was found between IL-10 (-C819T) polymorphism and HDA. Additionally, there was no difference in serum IL-10 levels between clinical groups.

Interleukin-10 is present in all individuals, but not at the same serum level, possibly due to the gene expression of the polymorphisms being different in each person.[12] Recently, Franco et al[13], 2021, evaluated 53 individuals without FPP and 16 patients with advanced FPP. Levels of IL-10 in peripheral blood mononuclear cell supernatants stimulated with soluble egg antigen were significantly elevated in patients with advanced FPP. The authors concluded that there is no difference in the IL-10 response to the soluble extracts of adult worms, indicating that cytokine production against the egg antigen does not follow a response pattern. In addition, the authors launched a hypothesis of trying to modulate IL-10 by fibrosis, since its levels are related to the thickening of the periportal space.

In addition, Silva et al9 (2014) evaluated three biallelic polymorphisms and putative genotype and haplotype frequencies of the IL-10 promoter gene in 203 individuals with EM in Pernambuco and found that the polymorphism (-C819T IL-10) was not associated with an advanced pattern of FPP in this population and possible development of HDA. The authors suggested future studies with larger samples to assess the association of the impact of other polymorphic variants of the IL-10 gene on disease severity.

On the other hand, Silva et al[14] (2016) also evaluated 119 individuals with EM and showed that none of the polymorphisms (-G1082A/C819T/-C592A) of the IL-10 gene were associated with FPP regression in this population. The authors suggested the need for studies with larger samples to better elucidate the impact of these polymorphisms on the pathogenesis of EM.

Brandt et al15, 2010, investigated the role of IL-10 and IL-13 cytokines in the development of hepatic fibrosis in three clinical groups: Group I - 25 patients with hepatosplenic schistosomiasis (EHE); Group II - 30 patients with EHE submitted to splenectomy and ligation of the left gastric vein; Group III - 33

individuals without EHE or any other disease or injury that would compromise liver function reserve. There was no significant difference between the mean IL-10 concentrations between the study groups, nor was there any difference between the serum concentrations of this cytokine and the presence of Symmers' fibrosis.

Additionally, Silva et al[9] (2014) also found that serum levels of IL-10 showed no association with the development of HDA. The authors suggest future cohort studies to assess the influence of IL-10 on the fibrogenesis of FPP and, consequently, HDA.

On the other hand, a cohort carried out in Uganda, evaluated 199 individuals, aged between 6 and 50 years, residents of a village located on the shores of Lake Albert, where it was found that a low amount of IL-10 was associated with FPP.16 The levels of nine immune molecules cells, including those of IL-10, were evaluated through blood samples. The data obtained revealed that children and adults had different factors associated with fibrosis. Most cases of fibrosis in children (eight out of nine) were associated with low factor IL-10 scores (< 47th percentile). The authors concluded that S. mansoni infections that result in periportal disease have a multifactorial molecular etiology. Additionally, they noted that women may be at a lower overall risk for FPP,

Arnaud et al (2008) evaluated serum levels of IL-10 in 58 individuals with mild FPP or without fibrosis compared to 27 individuals with severe FPP. And they found that individuals with severe fibrosis produced less IL-10 than individuals without fibrosis or mild fibrosis. The authors concluded that cytokines may be crucial for preventing disease in patients infected with S. japonicum eggs. Therefore, hypotheses are raised regarding the performance of IL-10 and the fundamental role in regulation, as observed in experimental models and suggested in studies of humans infected with S. mansoni. The results found in the present study do not exclude the possibility that other polymorphic variants of the gene IL-10 may influence the severity of HDA.

Therefore, future studies with larger samples are necessary to analyze this polymorphism and respective serum IL-10 levels, to better evaluate the impact of the (-C819T) IL-10 polymorphism and the IL-10 expression and FPP intensity and consequently HDA.

In summary, the results suggest that the -C819T polymorphism and IL-10 levels were not associated with HDA in this population. When considering the limitations imposed by the sample size of the present study, additional research is recommended to explore associations between this polymorphism and HDA.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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