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EVALUATION OF THE -C592A PROMOTER POLYMORPHISM AND PUTATIVE HAPLOTYPES OF THE INTERLEUCIN-10 (IL-10) GENE IN SCHISTOSOMIC HIGH DIGESTIVE HEMORRHAGE IN PERNAMBUCO

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Abstract: Introduction: Portal Hypertension is a complication of periportal fibrosis in the terminal branches of the hepatic portal vein in schistosomiasis individuals that causes splenomegaly and the formation of varicose veins. The rupture of varicose veins causes Upper Digestive Bleeding (UDH). Single Nucleotide Polymorphisms and putative Haplotypes of the Interleukin-10 (IL-10) gene may be involved in modulating the immune response and development of the disease. **Goal:** To investigate the association between the polymorphism in the –C592A IL-10 region and the putative haplotypes of the IL-10 gene with the severity of UGIB in individuals with Schistosomiasis Mansoni treated at the Gastroenterology outpatient clinic at the Hospital das Clínicas of ``Universidade Federal de Pernambuco`` (HC-UFPE) in Pernambuco. Method: This is a cross-sectional study, which involved 124 individuals infected with Schistosoma mansoni, after specific treatment, to verify the association between the –C592A IL-10 genetic polymorphism and the putative haplotypes of the IL-10 gene with HDA. **Results:** There was no evidence of a statistically significant association between the - 592A IL-10 polymorphism and UGIB between the clinical groups (PR = 0.803, 95% CI = [0.29-1.25] and p = 0.120). There was no evidence of a statistically significant association between the putative haplotypes (-G1082A/-T819C/-C592A) IL-10 and UGIB. **Conclusions:** The results suggest that the IL-10 -C592A polymorphism and putative IL-10 promoter gene haplotypes were not associated with UGIB in this population.

Key words: Schistosomiasis mansoni, Interleukin-10, Periportal fibrosis, Genetic polymorphism, Upper gastrointestinal bleeding.

INTRODUCTION

Schistosomiasis Mansoni (MS) is a neglected disease that affects around 240 million people worldwide (Rodrigues et al., 2022). In Brazil, the parasitosis is caused by Schistosoma mansoni and is considered endemic in the state of Pernambuco, with cases reported in 102 of its 185 municipalities (Rodrigues et al., 2022). It is a public health problem associated with the disease-impoverishment-poverty cycle, and its epidemiological profile is determined by the interconnection of ecological, biological, socioeconomic and cultural factors of individuals (Melo et al., 2019).

In the acute phase of MS, the cellular immune response leads to the formation of inflammatory granulomas around the parasite eggs that are deposited in the host's organs and tissues (Cortés et al., 2022). In the chronic phase of infection, granulomas decrease in size and are replaced by fibrotic tissue, compromising the function of the affected organ (Cortés et al., 2022). In the latter, the disease can manifest itself in two main clinical forms: Hepatointestinal Schistosomiasis (HIE) and Hepatosplenic Schistosomiasis (HEH) (Masi et al., 2020).

The HIE affects up to 90% of schistosomiasis individuals, and consists of granulomatous inflammation in the liver and intestine, with the absence or discrete presence of hepatosplenomegaly, periportal fibrosis (PPF) and Pulmonary Hypertension (PH) (Roriz et al., 2021). The parasite's eggs accumulate in the intestinal mucosa, mainly in the colon and rectum, and cause a granulomatous inflammatory reaction, ulcers and fibrosis (Carbonell et al., 2021).

EHE, a severe form of MS, occurs in up to 10% of infected patients and is characterized by hepatosplenomegaly (Masi et al., 2020). The chronic granulomatous immune response in liver tissue leads to PPF in the terminal branches of the hepatic portal vein, the main complication of which is PH, which causes splenomegaly and the formation of esophageal, gastric, splenorenal, pencreaticoduodenal and periumbilical varices (Cortés et al., 2022; Masi et al., 2020).

The rupture of gastroesophageal varices causes Upper Digestive Bleeding (UDH), which is potentially fatal (Cortés et al., 2022; Gunda et al., 2020). The severity of MS symptoms was associated with the cytokines participating in the granulomatous response, including Interleukin-10 (IL-10) (Marume et al., 2020). IL-10 is an anti-inflammatory and anti-fibrotic cytokine that acts in the control and modulation of inflammation and hepatic fibrogenesis through autocrine and paracrine mechanisms (Steen et al., 2020; Eibaky et al., 2020).

Single Nucleotide Polymorphisms (SNPs) can influence gene or protein expression, and may therefore be involved in modulating the immune response and disease development (Franco et al., 2021). One of the SNPs located in the promoter region of the IL-10 gene is -C592A-, which consists of replacing a Cytosine (C) nucleotide with an Adenine (A) nucleotide in region 592 (Adedokun et al., 20218). The A and C alleles at position -592 are in linkage disequilibrium with Thymine (T) and C in the -819-promoter region and are inherited together (-819C/-592C and - 819T/-592A) (Silva et al, 2016; Turner et al., 1997).

The combination of alleles from different regions of the IL-10 gene (-G1082A/-C819T/-C592A) form the putative haplotypes GCC, ACC, ATA and GTA (rare) (Silva et al., 2014; Turner et al., 1997). Thus, the present study evaluated the association between the (–C592A-) IL-10 polymorphism and putative haplotypes of the IL-10 gene with the severity of UGIB in schistosomiasis individuals in Pernambuco, Northeast Brazil.

GOALS

To investigate the association between the polymorphism in the –C592A- IL-10 region and the putative haplotypes of the IL-10 gene with the severity of UGIB in individuals with Schistosomiasis Mansoni treated at the Gastroenterology outpatient clinic at the Hospital das Clínicas of the ``Universidade Federal de Pernambuco`` (HC- UFPE) in Pernambuco.

METHODOLOGY

This is a cross-sectional study, with comparison between clinical groups, with 124 individuals infected with S. mansoni, aged 18 years or over, treated at the 3 Gastroenterology Outpatient Clinic of the Hospital das Clínicas of ``Universidade Federal de Pernambuco`` (HC -UFPE), to verify associations between immunogenetic factors and UGIB.

The individuals treated came from areas endemic for Schistosomiasis, infected by S. mansoni and had an epidemiological history and specific treatment for MS. Participants were divided into two exposure groups: GROUP 1 - With 68 individuals with EHE, with advanced FPP (Pattern E or F) and with UGIB; and GROUP 2 - with 56 individuals with HIE, with mild PPF (Pattern C) or without fibrosis (Pattern A) and without UGIB (Figure 1).

Individuals with EHE in the advanced phase (Pattern E or F according to the Niamey Classification), with UGIB and splenomegaly or a history of previous splenomegaly were included, based on abdominal USG confirmation; and individuals with HIE, mild PPF (Pattern C according to the Niamey classification) or without fibrosis (Pattern A) and without UGIB. (Richter et al., 2001).

Individuals with other liver diseases were excluded, such as liver cirrhosis, steatosis, hepatitis B or C and alcoholic disease, which were ruled out based on clinical history, abdominal US and specific laboratory tests (HBsAg, anti-HBc, anti-HBs and anti-HCV). ; patients undergoing blood transfusion within a period of less than three months; other already diagnosed clinical forms of schistosomiasis, such as pulmonary vascular disorders, pseudoneoplastic forms, schistosomiasis and medullary nephropathy. Individuals with a moderate pattern (Pattern D according to the Niamey Classification) of HGS were also excluded.

The steps related to the diagnosis of clinical forms of Schistosomiasis, biological samples, processing of biological material, molecular biology method and genotypic categorization have already been carried out in the period from 2012 to 2013, since this is a subproject of the umbrella project "EVALUATION OF THE SINGLE BASE POLYMORPHISMS OF THE MBL2, IL-10 AND TNF-α GENES IN THE IMMUNE RESPONSE TO PERIPORTAL FIBROSIS" (SILVA, P.C.V. 2014), with financial support from the notice Research Group/PROPESQ, 2014, under process number 038882/ 2014-10.

The determination of the –C592A IL-10 polymorphism was carried out at the Molecular Biology Laboratory of the Pediatric Oncohematology Center of the ``Universidade de Pernambuco`` (CEONHPEUPE), using PCR-RT (Polymerase Chain Reaction). (Sambrook; Russell, 2001; kube et al, 2003). The frequency of putative haplotypes (GCC/ ACC/ATA/GTA) was determined using ARLEQUIN software version 3.5.1.2 (Bern, Switzerland).

For this study, the association of the –C592A IL-10 polymorphism and putative haplotypes of the IL-10 gene with the severity of schistosomal UGIB was analyzed through analysis of the epidemiological and immunogenetic database of the "Clinical and Experimental Schistosomiasis" research group, in 2022.

The collected data were tabulated in double entry and sampling was done using Prevalence Ratio (PR) and 95% confidence intervals (95% CI), which were performed by bivariate analysis, and investigated the relationship between genotypic frequencies of the polymorphism –C592A IL-10 and the putative haplotypes of the IL-10 gene with HDA in the selected individuals. The analysis was performed using Epi-Info software version 3.5.5 (CDC, Atlanta, GA, USA) and the results were considered significant when $p< 0.05$.

The present study received authorization from the Ethics and Research Committee of the Health Sciences Center, UFPE, under protocol 113.199 and CAAE 03161512.6.0000.5208.

RESULTS AND DISCUSSION

There was no evidence of a statistically significant association between the - 592A IL-10 polymorphism and UGIB between the clinical groups (PR = 0.803, 95% CI = [0.29-1.25] and p = 0.120) (Table 1).

Silva et al (2014) evaluated the association of SNPs (-1082/-592/-819) IL-10 and putative haplotypes of the IL-10 promoter gene with the severity of PPF in 203 individuals infected with S. mansoni and found no association between the -C592A IL-10 polymorphism and the advanced FPP pattern. The authors suggested carrying out new studies, with a larger sample size, to better evaluate the association and impact of other polymorphic variables of the IL-10 gene on the pathogenesis of MS.

In another study, Silva et al (2016) retrospectively followed, in a two-year cohort, 125 patients who underwent specific treatment for MS and found no association between IL-10 promoter gene polymorphisms and PPF regression in this population. For the authors, studies with larger samples are necessary to better understand the possible connections between the -G1082A/C819T/-C592A IL-10 polymorphisms, the expression of IL-10 and the intensity of PPF.

Constantino and collaborators (2022) did not find an association between the –C819T IL-10 genetic polymorphism and its serum concentrations with UGIB in their study with 123 individuals infected with S. mansoni. Considering the sample limitation of their study, the authors recommended additional research to explore the associations between IL-10 gene polymorphisms and UGIB in individuals with MS.

There was no evidence of a statistically significant association between the putative haplotypes (-G1082A/-T819C/-C592A) IL-10 and UGIB (Table 2).

In the study by Silva et al., 2014, with 203 individuals with MS, a significant association was found between the putative ATA haplotype and the severity of PPF, while the ACC/GTA haplotypes were associated with a reduced risk of an advanced pattern of PPF. The study concluded that the putative ATA haplotype may be a risk and predictive factor for the severity of advanced PPF in the Brazilian population (Silva et al., 2014).

CONCLUSIONS

The results suggest that the -C592A- IL-10 polymorphism and the putative haplotypes of the IL-10 promoter gene were not associated with UGIB in this population. 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 on the prediction of schistosomiasis UGIB.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FINANCING

Research group/PROPESQ, 2014, under process number: 038882/2014-10.

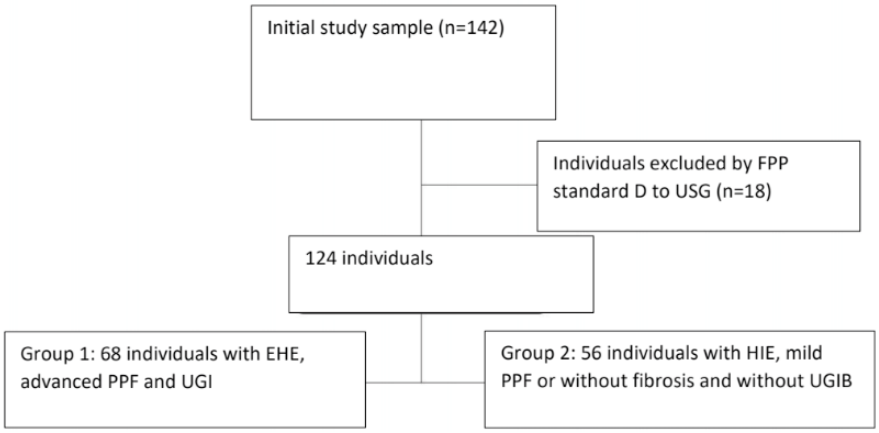

Figure 1: Participant eligibility flowchart.

	HDA						
	Yes		No				
Polymorphism-C592A *IL-10**	**n**	**%**	**n**	**%**	**RP**	**IC 95%**	**p-value**
1.AA/CA	35	51,4%	35	63,6%	0,803	[0,29-1,25]	0,120
2.CC	33	48,6	20	36,4%			
Total	68	100%	55	100%			

Table 1: Association analysis between the -C592A IL-10 polymorphism and HDA, Pernambuco, 2023.

*123 participants were evaluated for the determination of the –C59A IL-10 polymorphism through PCR-AE (Allele-Specific Polymerase Chain Reaction).

	HDA						
	Yes		No				
PUTATIVE HAPLOTYPES (-G1082A/-T819C/-C592A)	**n**	**%**	**N**	**%**	**RP**	**IC 95%**	**p-value**
GCC	5	7,8%	8	13,4%		(Referência)	
ACC	25	39%	19	35,8%	1,47	[0,708-3,078]	0,396
ATA	21	32,9%	10	18.8%	1,76	[0,849-3,652]	0,142
GTA	13	20,3%	17	32%	1,12	[0,506-2,507]	1
TOTAL	64	100%	53	100%			

Table 2: Association analysis between putative haplotypes (-G1082A/-T819C/-C592A) IL-10 and HDA, Pernambuco, 2023.

*117 Individuals participated in the construction of putative haplotypes by ARLEQUIM (Bern, Switzerland).

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