Evaluation of the cytokines IL-10 and IL-13 as mediators in the progression of symmers fibrosis in patients with hepatosplenic schistosomiasis mansoni

Avaliação das citocinas IL-10 e IL-13 como mediadores na progressão da fibrose de Symmers em portadores de esquistossomose mansônica na forma hepatoesplênica

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A B S T R A C T

Objective: To investigate the serum levels of IL-10 and IL-13 in patients with hepatosplenic schistosomiasis mansoni (HSM), evaluating the role of these cytokines in the development of hepatic fibrosis. **Methods**: The study was prospective and analytical, developed at the Department of Surgery, Federal University of Pernambuco, Keizo Asami Laboratory of Immunology. We studied three groups: Group I - 25 patients with hepatosplenic schistosomiasis mansoni who were not submitted to surgery; Group II - 30 individuals who underwent splenectomy and ligature of left gastric vein; Group III - 33 subjects without hepatosplenic schistosomiasis mansoni or any other disease or condition that could compromise the hepatic functional reserve. Serum concentrations of IL-10 and IL-13 were obtained through ELISA. Considering their non-parametric nature, all concentrations were analyzed by Kruskal-Wallis test, with $p<0.05$ used to reject the null hypothesis. **Results:** The mean concentrations of IL-10 in ng/mL in serum were GI: 50.0 ± 59.0; GII: 38.0 ± 270; GIII: 38.0 ± 20.0. Concentrations of IL-13 in ng/mL in the serum of patients were respectively: 41.0 ± 93.0 in GI, 16.0 ± 17.0 in GII and 18.0 ± 34.0 in GIII. There was no significant difference between the mean concentrations of IL-10 and IL-13 between the study groups ($p> 0.05$). **Conclusion**: The mean serum concentrations of IL-10 and IL-13 were similar in all three groups, indicating that possibly the presence of these cytokines in serum is not associated with different degrees of Symmers fibrosis in patients with hepatosplenic schistosomiasis mansoni.

Key words: Schistosomiasis mansoni, Spleen, Splenectomy, Interleukin-10, Interleukin

INTRODUCTION

Schistosomiasis is a disease that affects about 200 million people worldwide. In Brazil it is estimated that there are six million carriers[1]. Brazilian northeastern region is the one in which it is more prevalent[2]. In Pernambuco, the highest prevalence is in the Zona da Mata, often ranging from 10% to 50% of their habitants[3].

The disease presents in various clinical forms, with 5% to 7% of subjects developing serious lesions in liver and spleen, the fibrosis being an important aspect of the infection by *Schistosoma mansoni* in man[4]. Schistosomal fibrosis is caused by a cellular immune reaction (granuloma) directed against the parasite egg deposited in the liver. The newly formed granuloma is an inflammatory reaction and, when it is poorly controlled by the body, it becomes fibrotic. The formation of the granuloma is mediated primarily by $CD4^+$ T cells, endothelial cells, activated platelets, neutrophils and hepatocytes[5]. Fibrosis happens due to the accumulation of extracellular matrix proteins (ECMPs), for example, laminin, collagen and connectin in the portal space, a process known as periportal fibrosis (PPF)[6]. The ECMPs are produced mainly by the Ito liver cells after their differentiation into myofibroblasts[7]. In some patients the fibrous tissue can be replaced by normal tissue (fibrolysis). In this process there is Ito cells apoptosis and increased activity of metalloproteinases (enzymes that degrade ECMPs)[8]. However, some patients may display an imbalance between fibrogenesis and fibrolysis and therefore the develop PPF in its critical condition. In these individuals there is increasing accumulation of ECMPs due

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to lack of Ito cells apoptosis and accumulation of fibrous tissue around the intrahepatic portal vein branches[5].

Several studies were conducted to determine the role of certain cytokines in the regulation of fibrogenesis and fibrolysis. With animal models, some showed that the Th2 cytokines (IL-4, IL-5 and IL-13) contribute to granuloma formation and development of fibrosis[9]. These cytokines stimulate the proliferation of fibroblasts and production of ECMPs[10]. IFN-ã has been implicated in fibrolysis, in which it acts by inhibiting the proliferation of myofibroblasts and the production of ECMPs[8]. In humans, studies in endemic regions for *Shistosoma mansoni* demonstrate a protective role of IFN-ã and an aggravating one of TNF á in the control of severe fibrosis[11]. It was also shown that IL-10, IL-5 and IL-13 are strongly associated with severe forms of fibrosis[12]. Recently it was shown by multivariate analysis that IL-13 is the most strongly associated cytokine with the development of severe forms of fibrosis[13]. These studies were based on the production of cytokines in culture supernatants of peripheral blood mononuclear cells (PBMC) after stimulation with *S. mansoni* egg antigens. Recent studies suggest that regulatory T cells ($CD4^+$, $CD25^+$) that produce IL-10 may interfere with the protective immunity against *Schistosoma*[14].

Given the involvement of IL-13 and IL-10 in the development of Symmers fibrosis, this study aimed to evaluate the role of these cytokines in the development of liver fibrosis in patients with schistosomiasis. Thus, we compared the levels of IL-10 and IL-13 in serum between patients with hepatosplenic schistosomiasis mansoni (HSM), patients with HSM who underwent splenectomy and ligature of left gastric vein and patients without HSM or any disturbance to the hepatic functional reserve.

METHODS

The study was prospective and analytical, conducted in the Department of Surgery, Federal University of Pernambuco and in the Keizo Asami Immunology Lab (LIKA). The project was approved by the Ethics in Research Committee (CEP) of the Health Sciences Center. Study volunteers signed the consent form, after which they were included in the investigation.

Blood collection was performed at the time of exams in the Hospital das Clinicas of the Center for Health Sciences, Federal University of Pernambuco. The study population consisted of three groups: Group I - 25 patients with hepatosplenic schistosomiasis mansoni (HSM), adults of both genders (16 female and nine male), aged between 15 and 80 , who had not undergone surgical treatment; Group II - 30 patients with HSM who had been submitted to splenectomy and ligature of left gastric vein, adults of both sexes (19 females and 11 males), aged between 12 and 77 years; and Group III - 33 volunteer subjects without HSM or any other disease or condition that could compromise hepatic functional reserve, coming from the same endemic area and having the same socioeconomic status, adults of both sexes (25 females and eight male), aged between 13 and 40 years (Figure 1).

Exclusion criteria were: patients with hematologic diseases, individuals with positive tests for hepatitis B and / or C, patients with a history of alcohol abuse and / or liver biopsy with lesions suggestive of alcoholic liver disease, patients under treatment with corticosteroids or immunosuppressors or immune system stimulants during the study period.

Cytokine measurements were made by collecting samples of 5 ml of peripheral blood from the three groups. The blood was distributed in dry tubes. The collected materials were centrifuged at 3000 rpm for five minutes. After centrifugation, the sera were placed in plastic and sterile Eppendorf tubes, and stored at -20° C for later measurement of interleukins. Serum concentrations of IL-10 and IL-13 were obtained using the respective Quantikine® kit (R & D systems).

In analyzing the results, graphs were made using SPSS. Statistical differences between groups were analyzed using the Jandel Sigma Stat software version 2.0. Considering their non-parametric nature, all concentrations were analyzed by Kruskal-Wallis test. Statistical significance was considered at a critical level of 5% in all cases ($p \pounds 0.05$) to reject the null hypothesis.

RESULTS

We evaluated 25 patients with HSM (Group I - GI), 30 patients with HSM who underwent splenectomy and ligature of left gastric vein (Group II - GII) and 33 patients without HSM or any harm to their hepatic functional reserve (Group-III - GIII) . The mean concentrations of IL-10 in ng/ml in serum were 50.0 ± 59.0 in GI, 38.0 ± 27.0 in GII and 38.0 ± 20.0 in GIII. There was no significant difference ($p>0.05$) between the mean concentrations of IL-10 between the study groups (Figure 2A). When comparing the mean IL-10 between Groups I and II, there was no difference between serum concentrations of cytokines and the presence of Symmers fibrosis ($p>0.05$).

The mean concentrations of IL-13 in ng/ml in the serum of patients were respectively: 41.0 ± 93.0 in GI, 16.0 ± 17.0 in GII and 18.0 ± 34.0 in GIII. Similarly, there was no significant difference ($p>0.05$) between the mean concentrations of IL-13 between groups and also with the presence of Symmers fibrosis in treated patients (Figure 2B). It was observed that the mean serum concentrations of IL-13 in Group I (untreated patients - HSM) tended to be higher when compared with the isolation of the control (GIII) and the treated (GII) groups. Likewise, when we compared the mean IL-13 between groups I and II, there was no association of this cytokine and the presence of Symmers fibrosis ($p> 0.05$).

Works undertaken in endemic areas show the existence of a relationship between prolonged exposure to the parasite, intensity of the infection and progression to the severe form of fibrosis[15]. However, other studies show that the severity of fibrosis does not always correlate with the levels of infection, with advanced stages of fibrosis in low transmission areas[16], or high levels of infection in patients who do not develop fibrosis[17].These results can be explained by the fact that periportal fibrosis is a chronic disease that develops after several years of exposure to the parasite. It may have been diagnosed at a time that infection levels are low, but as a result of a high level of infection years before diagnosis. This is confirmed by the fact that 88.0 and 55.2% of patients in Group I and II, respectively, were aged above 40 years (Figure 1B).

DISCUSSION

Hepatosplenic Schistosomiasis is a multifactorial disease whose development depends on the interaction of various environmental factors and the host. Genetic studies have demonstrated the existence of resistant and susceptible individuals to infection by *S. mansoni*; they also demonstrated that the mechanisms of immune protection against infection or against the form of severe liver disease are distinct. Consequently, pathways involved in immunological protection and in aggravating the disease have been identified. These studies may uncover the pathways that should be used as targets for vaccines, leading tests to be made in order to protect individuals from infection. Susceptibility alleles may also be used to identify individuals who have high risk/likelihood of developing the infection in its severe form, and these individuals should be included in vaccination or protected with regular chemotherapy.

An important role of interleukin 10 and 13 in the development of liver fibrosis has been shown in studies conducted in mice, and in humans it has been investigated worldwide[18,19]. However, there were no significant differences between the concentrations of IL-10 and IL-13 in the serum of patients with HSM (Group I), the ones with HSM who underwent splenectomy and ligature of left gastric vein (Group II) and volunteers without HSM or any other disease or condition that could compromise hepatic function (Group III). Further studies should be performed to quantify these cytokines in culture supernatants after PBMC stimulation with egg antigens and the *Schistosoma* parasite. In addition, there could be quantification of the the expression of IL10 and IL13 genes in liver biopsies and PBMC after stimulation with parasite antigens. Branding studies of different populations of lymphocytes by flow cytometry may also be conducted. Also, studies with other cytokines involved in the Th1, Th2 and Th17 pathways might play an important role.

Epidemiological studies conducted in a highly endemic area showed that patients with severe fibrosis are more common in certain families, suggesting the involvement of hereditary factors in the development of this disease. With the goal of finding the gene (or genes) associated with susceptibility/resistance to schistosomiasis fibrosis, several genetic studies have been carried out. Association was found between the levels of infection and the region 5q31 q33[20], in which the IL13 gene is located. Polymorphisms in the promoter region of this gene were associated with high frequency of infection[21].Studies in patients with severe periportal fibrosis associated with

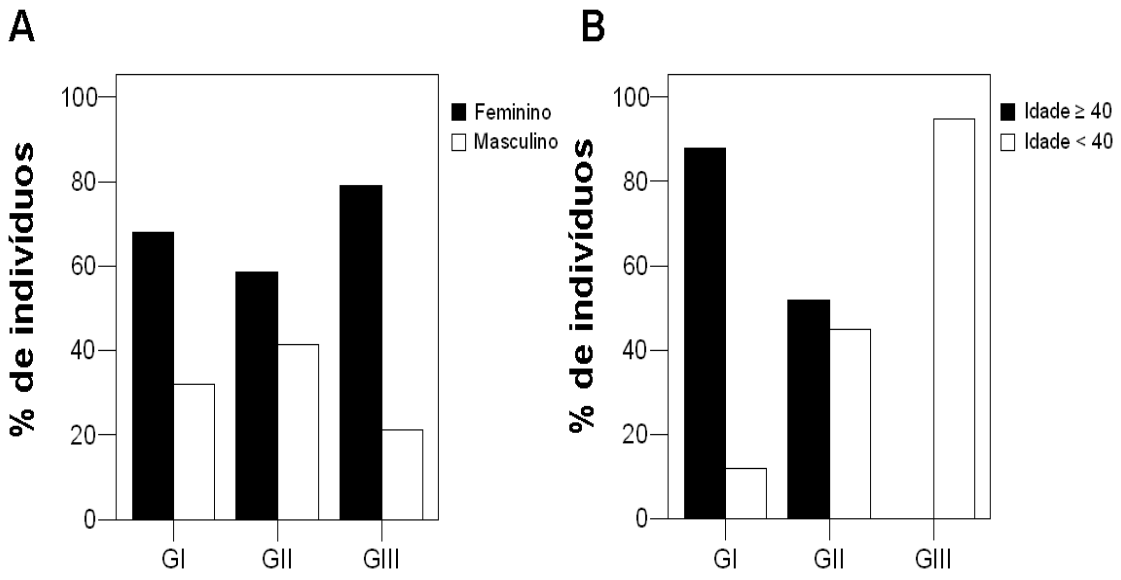

Figure 1 - *Study samples characteristics: A, percentage of individuals by sex in groups of patients; B, percentage of individuals by age in each group.*

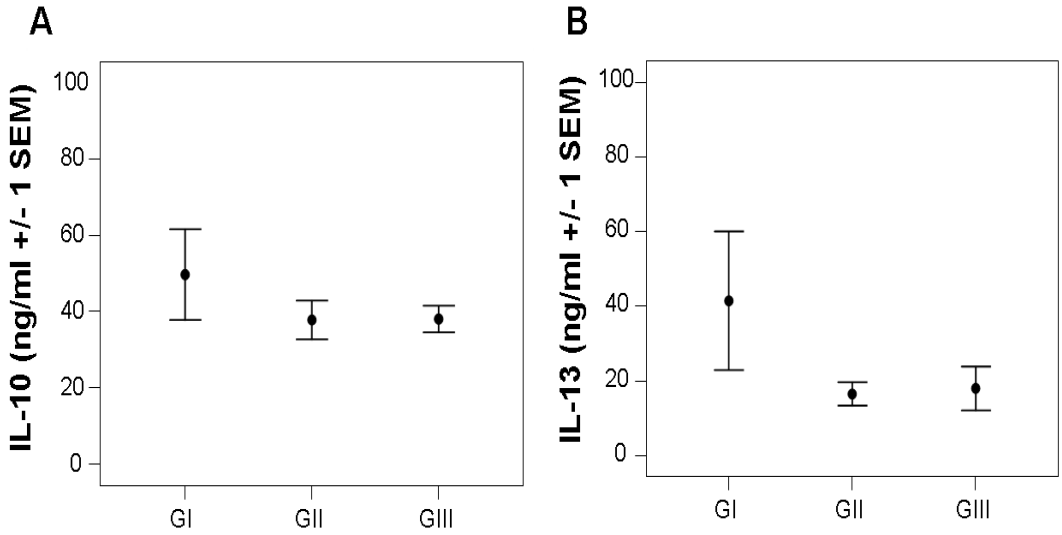

Figure 2 - *Production of IL-10 (A) and IL-13 (B) in patients from Groups I, II and III.*

portal hypertension detected significant association of regions 6q22-q23[22] and 12q24[23] in the susceptibility/ resistance to developing this phenotype. Polymorphisms in the genes IFNGR1 (6q22-q23), which codes the á chain of the IFN-ã receptor[22] and IFNG (12q24)[23] were found to be associated as well.

A recent genetic study showed that the Connective Tissue Growth Factor (CTGF) gene is associated with schistosomal fibrosis. This gene was shown to be implicated in numerous cellular functions: growth, proliferation, apoptosis, adhesion, migration, extracellular matrix production and differentiation[24], besides being involved in the fibrotic mechanism of various diseases such as lung, heart, liver and periportal schistosomal fibrosis[25-27]. These studies open new perspectives for diagnosis, since the Single-nucleotide Polymorphism (SNP) highlighted in this gene could be used in the form of a kit, focused on the detection of patients with genetic predisposition to the development of severe forms of fibrosis caused by the parasite.

In this context, it is clear that clinical, immunological and genetic factors are of great importance for the development of new diagnostic and therapeutic approaches.

In conclusion, mean serum concentrations of IL-10 and IL-13 were similar in all three groups, indicating that possibly these cytokines are not associated with different degrees of Symmers fibrosis in patients.

R E S U M O

Objetivo: *Investigar os níveis de IL-10 e IL-13 no soro de portadores da esquistossomose mansônica na forma hepatoesplênica (EHE), avaliando o papel destas citocinas no desenvolvimento da fibrose hepática.* ***Métodos:*** *O estudo foi prospectivo e analítico, desenvolvido no Departamento de Cirurgia da Universidade Federal de Pernambuco, Laboratório de Imunologia Keizo Asami. Foram estudados três grupos: Grupo I - 25 portadores de esquistossomose mansônica na forma hepatoesplênica e não submetidos a tratamento cirúrgico; Grupo II - 30 submetidos à esplenectomia e ligadura da veia gástrica esquerda; Grupo III - 33 indivíduos sem esquistossomose mansônica na forma hepatoesplênica ou qualquer outra doença ou agravo que comprometesse a reserva funcional hepática. As concentrações séricas de IL-10 e IL-13 foram obtidas pelo método ELISA. Considerando-se a natureza não paramétrica, todas as concentrações foram analisadas pelo teste de Kruskal-Wallis. p<0,05 foi usado para rejeição da hipótese de nulidade.* ***Resultados:*** *As médias das concentrações de IL-10, em ng/mL, no soro foram: GI 50,0 ± 59,0; GII 38,0 ± 270; GIII 38,0 ± 20,0. As concentrações de IL-13, em ng/mL, no soro dos pacientes foram respectivamente: GI 41,0 ± 93,0; GII 16,0 ± 17,0; GIII 18,0 ± 34,0. Não se observou diferença significante entre as médias das concentrações de IL-10 e IL-13 entre os grupos de estudo (p>0,05).* ***Conclusão:*** *As médias das concentrações séricas de IL-10 e IL-13 foram similares nos três grupos estudados, indicando que, possivelmente, estas citocinas no soro não estejam associadas aos diferentes graus de fibrose de Symmers nos pacientes.*

Descritores: *Esquistossomose mansoni. Baço. Esplenectomia. Interleucina-10. Interleucina-13.*

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