Morphometric analysis of liver and spleen type I and III collagen of autopsied patients with acquired immunodeficiency syndrome

Análise morfométrica do colágeno tipo I e III do fígado e baço de pacientes autopsiados com a Síndrome da Imunodeficiência Adquirida

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ABSTRACT

Acquired Immunodeficiency Syndrome (AIDS) is caused by the Human Immunodeficiency Virus (HIV), a retrovirus capable of invading and replicating in host leucocytes, favoring installation of opportunistic infections and causing important changes in organs such as spleen and liver. The aim of this study was to quantify percentage of type I and III collagen in the spleen and liver of autopsied patients divided in two groups: with AIDS (n=24) and without AIDS (n=24). Quantification of type I and type III collagen was made with Sirius Red and Reticulin stains, respectively, using the image analysis system Leica Qwin Plus®15. From medical records, we retrieved information regarding age, sex, race, Body Mass Index, and spleen and liver weight. We analyzed the data with SigmaStat® 2.03, considering statistically significant values of p<0.05. Patients with AIDS showed higher percentage of type I and type III collagen, with significant difference only between type III in both organs. The virus contributes with increase the amount of type I and type III collagen in liver and spleen of HIV infected patients contributing to morphofunctional changes. These changes may be related to decline of immune function due to viral presence and the increase of these fibers in those organs.

Keywords: AIDS, autopsy, spleen, liver.

1. INTRODUCTION

The Human Immunodeficiency Virus (HIV), a retrovirus capable of invading and replicating in host defense cells, especially in CD4 lymphocytes, causes Acquired Immunodeficiency Syndrome (AIDS). The syndrome is characterized by CD4 lymphocytes count below 350 cells/mm³ and elevated plasma viral RNA levels...
When treatment does not occur early, the probability of developing AIDS increases, favoring the onset of opportunistic infections and causing important changes in several organs with primordial functions, such as spleen and liver (BRASIL, 2016).

Secondary lymphoid organs are responsible for the immune response and for the maintenance of the necessary amount of T cells, B cells and antigen presenting cells (BOASSO et al., 2007; ABBAS, LICHTMAN, PILLAI, 2015). Studies in other lymphoid organs, such as palatine tonsils, have shown that HIV-infected patients have a higher percentage of collagen fibers, especially type I collagen, compared to uninfected ones, and this architectural change may be related to disturbances in the immune function of lymphoid tissues (ZENG et al., 2012; BEGHINI et al., 2015). Extracellular matrix of the organ is constituted by collagen type III among others and its function is structural support (JUNQUEIRA, CARNEIRO, 2017), however there are no studies approaching this specific collagen type in HIV-infected patients.

Liver promotes processing and storage of absorbed nutrients from small intestine, detoxification, synthesis of plasma proteins and bile. Liver disease is prevalent among HIV-infected individuals and the organ is considered a viral reservoir as the virus is able to replicate within Kupffer cells in the beginning of infection (MACÍAS et al., 2016; SWANSON et al., 2016). Studies suggest a direct relationship between viral infection and fibrosis as virus directly infects hepatocytes, stimulating the production of proinflammatory cytokines and consequently favoring collagen neoformation (SWANSON et al., 2016). In addition, the use of antiretrovirals, alcohol abuse, obesity and immunosuppression are other factors that contribute to changes in extracellular matrix components (MACÍAS et al., 2016).

Despite spleen and liver importance, there are still few reports in the literature about changes that HIV causes in these organs, especially in autopsy material. Therefore, the objective of this study is to analyze the percentage of type I and type III collagen in spleen and liver from autopsied patients with AIDS.

2. MATERIALS AND METHODS

Research Ethics Committee of the Federal University of Triangulo Mineiro approved the present study (number 46251515.5.0000.5154).

This is a retrospective study, in which we evaluated about 400 protocols from autopsied patients between 18 and 50 years old, conducted between 1996 and 2017 in the
General Pathology Discipline at the Clinical Hospital of the Federal University of Triângulo Mineiro (HC-UFTM), Uberaba, Minas Gerais, Brazil. Of these, we selected 24 patients with AIDS and 24 without AIDS, matched by age, gender and race. Data about age, gender (female and male), race (white or non-white), body mass index (BMI), and spleen and liver weight were obtained from autopsy protocols. BMI values between 18.5 to 24.9 kg/m² were considered normal, below 18.5 kg/m² considered undernourished and between 25.0 e 29.9 kg/m², overweight (BARBOSA, FORNÉS, 2013).

After macroscopic analysis, fragments, which were already fixed in 10% formaldehyde, were submitted to dehydration in increasing concentration of alcohol (70% to 100%), diaphanized in xylol and included in paraffin. Fragments were submitted to histological processing and serially sectioned with 4μm thickness. Sirius Red (SR) and Reticulin (RET) stains were performed for histochemical analysis.

Slides stained with SR were examined under polarized light using a 40x objective, and the area composed of type I collagen presented a birefringent appearance with a reddish-yellow coloration. Slides stained by RET were analyzed under common light, and the area consisting of type II collagen showed blackish coloration.

We analyzed 60 microscopic fields, defined by the calculation of Cumulative Average. Quantification was performed using the image analyzer system Leica QWin Plus®15 (Cambridge, UK), in which type I and III collagen were marked to obtain the percentage by field area analyzed.

Statistical analysis was performed using SigmaStat® 2.03. In cases of normal distribution and similar variances, Student's t-test was used for comparison of two groups, values were expressed as mean ± standard deviation (x ± SD). In cases of non-normal or normal distribution with non-similar variances, a non-parametric test, Mann-Whitney (T), was used, followed by the Dunn test when needed. In this type of distribution, values were expressed in median and minimum and maximum values (Med-Min-Max). Results were considered statistically significant when p <0.05.

3. RESULTS

The mean age of patients with AIDS included in this study was 35.12 ± 6.82 years, ranging from 23 to 48 years old. Among non-AIDS patients, this mean was 35.67 ± 7.68, ranging from 20 to 49 years old (p <0.79). In both groups, 13 (54.17%) patients were female and 15 (62.5%) were white. The mean BMI of patients with AIDS was 19.71 kg/m² and in patients without the syndrome was 25.06 kg /m², and this difference was
statistically significant. Patients with AIDS presented higher hepatic and splenic weight, but without significant difference (Table 1).

Table 1. Body mass index (BMI), hepatic and spleen weight of 48 patients with and without AIDS autopsied in HC-UFTM from 1996 to 2017.

|                  | BMI (kg/m²) | Liver weight (g) | Spleen weight (g) |
|------------------|-------------|------------------|-------------------|
|                  | Med (Min-Max) | (x ± SD)* | Med (Min-Max) |
| With AIDS        | 19.71 (1.60-33.80) | 1846.45 ± 559.63 | 215.00 (44.00-915.00) |
| Without AIDS     | 25.06 (16.32-38.30) | 1668.08 ± 606.14 | 187.50 (30.00-960.00) |
| T                | T=406.000    | t=1.059         | T=611.000         |
| p                | p<0.003      | p<0.295         | p<0.643           |

*x ± SD: media and standard deviation; “t”: Student test; T: Mann Whitney test; p<0.05.

In hepatic fiber analysis, patients with AIDS had a higher percentage of type I and III collagen than those without the syndrome, but with significant difference only among the first (Table 2, Fig. 1A-D).

Figure 1. Percentage of type I and III collagen in the liver from 48 autopsied patients with and without AIDS of HC-UFTM from 1996 to 2017. A) Higher percentage of type I collagen in the liver of patients with AIDS (SR, 40x). B) Lower percentage of type I collagen in the liver of patients without AIDS (SR, 40x). C) Higher percentage of type III collagen in the liver of patients with AIDS (RET, 40x). D) Lower percentage of type III collagen in the liver of patients without AIDS (RET, 40x).

In splenic fibers analysis, the group with AIDS had a higher percentage type I and III collagen, and there was a significant difference only between the latter (Table 2, Fig. 2A-D).
Figure 2. Percentage of type I and III collagen in the spleen from 48 patients with and without AIDS autopsied in HC-UFTM from 1996 to 2017. A) Higher percentage of type I collagen in the spleen of patients with AIDS (SR, 40X). B) Lower percentage of type I collagen in the spleen of patients without AIDS (SR, 40x). C) Higher percentage of type III collagen in the spleen of patients with AIDS (RET, 40x). D) Lower percentage of type III collagen in the spleen of patients with AIDS (RET, 40x).

Table 2. Percentage of type I and III collagen in the liver and spleen from 48 patients with and without AIDS autopsied in HC-UFTM from 1996 to 2017.

|                  | Type I collagen in the liver (%) | Type III collagen in the liver (%) | Type I collagen in the spleen (%) | Type III collagen in the spleen (%) |
|------------------|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| **With AIDS**    | Med (Min-Max)                     |                                   |                                   |                                   |
|                  | 4.60 (0.00-32.82)                 | 8.43 (0.65-25.21)                 | 3.40 (0.04-39.55)                 | 4.89 (0.01-34.32)                 |
| **Without AIDS** | 3.39 (0.02-88.09)                 | 7.93 (1.22-25.93)                 | 3.32 (0.00-32.38)                 | 3.99 (0.01-23.55)                 |
| **T**            | T=2254077.000                     | T=2109684.000                     | T=2104727.000                     | T=2228751.000                     |
| **p**            | p<0.001                           | p<0.113                           | p<0.173                           | p<0.001                           |

T: Mann Whitney test; p<0.05

4. DISCUSSION

HIV infection can influence the body in many ways, including its nutritional status. In this study, we can observe a significant difference between BMI of patients with and without AIDS, indicating that the disease influences in BMI decrease. In addition, factors such as opportunistic infections and the use of antiretroviral therapy also contribute to changes in nutritional status of patients with AIDS (BARBOSA, FORNÉS, 2013).

Hepatic and splenic abnormalities in HIV patients may be related to the decline of immune system function and of viral presence in cells of these organs, resulting in
cicatricial processes due to the intense inflammatory process. Changes in the amount of fibers can result in changes regarding structural organization, resistance and consistency of the liver and spleen (TUYAMA et al., 2010).

Increased collagen fibers, specially type I collagen, is related to HIV ability to infect stellate or Ito cells as these cells act as central mediators in hepatic fibrosis (BRUNO et al., 2010; TUYAMA et al., 2010). Some evidences suggest that the accumulation of these fibers in lymphocyte tissues in HIV patients contributes to the loss of lymphoid cells and loss of organ architecture, as well as a decrease in their immune function (SCHACKER et al., 2002; SCHACKER et al., 2006).

HIV-infected patients showed increase type III collagen, however, there was no evidence in the literature that justifies this increase. We believe that the virus is capable of interfering with this synthesis by the same mechanism above mentioned that contributed to the increase of collagen fibers.

There are reports of patients with AIDS using antiretroviral therapy that had lower amount of fibers compared to those who do not use it and a larger amount compared to patients without the disease, suggesting that even at small replication rates, the virus contributes to fibrosis in lymphoid tissues and prevents the restoration of their immune function (SANCHEZ et al., 2015; ANADOL et al., 2018).

Although our study did not evaluate antiretroviral therapy due to lack of information in medical records, some studies indicate that at the beginning of the infection, therapy may influence the formation of hepatic and splenic fibrosis, reducing the amount of fibers in these organs, which is a possible explanation for the non-significant differences of our study (OLEGARIO et al., 2011).

5. CONCLUSION

The relevance of this study was to characterize hepatosplenic changes of autopsied patients with AIDS. Data from this research reinforce the relevant observation that HIV increases the amount of type I and III collagen in the liver and spleen of infected patients. Increased knowledge about hepatic and splenic abnormalities regarding HIV can influence the progress of existing therapies and consequently improve quality of life of patients with AIDS.
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