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Original Article

# Reduction of immunity in HIV-infected individuals: Can fibrosis induce hypoplasia in palatine and lingual tonsils of individuals with HIV infection?

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ABSTRACT

The role of tonsils in oral immunity has been described. However, the pathogenesis of HIV infection in these organs is still unclear. The aim of this study is to perform histological and immunohistochemical analysis of the palatine and lingual tonsils of autopsied individuals with or without HIV infection. Twenty-six autopsied individuals with HIV infection (HI) ($n = 13$) and without HIV infection (CO) ($n = 13$) were selected. Palatine and lingual tonsil fragments were collected for histological and immunohistochemical analysis. We found in the HI group a higher frequency of hyaline degeneration in both palatine and lingual tonsils; smaller follicle areas, and a higher percentage of collagen in comparison with the CO group. In the HI group, there was higher density of blood vessels in palatine tonsils than in the CO group. In the HI group, there were significant positive correlations between palatine and lingual tonsils and the area of lymphoid follicles, and between the percentage of blood vessels and collagen in palatine tonsils. In addition, there was a significant negative correlation between the percentage of collagen and lymphoid follicle area in both palatine and lingual tonsils in the HI group. These findings suggest that the immune functions of these tonsils are prejudiced by fibrosis. Therapies to reduce the neoformation of collagen are required to improve immune function of organs against pathogens.



## Introduction

The human immunodeficiency virus infection (HIV infection), first described in 1981 [1], continues to be a serious public health problem in the world [2]. The human immunodeficiency virus (HIV), which causes Human Immunodeficiency Syndrome (AIDS), invades the host cell defenses, especially CD4+ T cells, causing immunosuppression [2].

In the acute phase of HIV infection, the secondary lymphoid organs show follicular hyperplasia and proliferation of vascular stroma [3]. At this stage, the germinal centers (GCs) of these organs are important reservoirs of free virus in the interstitial space [4]. As the disease progresses, the GCs suffer lysis [4] and involute [5–7], triggering depletion of CD4+ T cells [8–11]. Furthermore, during the progression of the disease, it has been demonstrated that collagen in the palatine tonsils of individuals with HIV infection increases compared with those individuals without HIV infection. It has also been shown that individuals who had higher collagen deposition in the tonsils also showed greater depletion of CD4+ T cells in these secondary lymphoid organ [12]. It is known that naive CD4+ T cells are dependent on organized lymphatic tissue to facilitate movement both in contact with the histocompatibility complexes and with growth factors necessary for their survival [13–15]. The progressive deposition of collagen can occupy up to a third of the area of the T cell deposition zone, limiting the physical space that T cells can occupy. Therefore, the architectural disruption of the normal T cell zone by fibrosis can induce depletion of naive CD4 T cells, with consequent depletion of T cells in the peripheral blood and lymphoid tissue. However, studies that support that theory are scarce [16].

Considering that tonsils are the lymphoid organs most affected by HIV [3], we believe that the histological changes found in the lingual tonsils of individuals with HIV infection could reflect the degree of disease progression. Besides this, HIV's effect on the tonsils could reduce local immune response, contributing to the

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**Table 1**
Data demographics of HI group and CO group.

| | Individuals of HI group ($n = 13$) | Individuals of CO group ($n = 13$) |
|---|---|---|
| Ethnicity[a] (C/NC) | 7:6 | 9:4 |
| Gender[b] (M/F) | 9:4 | 7:6 |
| Age[c] (years, means ± SD) | 39.31 ± 2.610 | 44.85 ± 2.928 |

C: Caucasian; NC: non-Caucasian; M: male; F: female; SD: standard deviation.
[a] Exact Fisher test, $p = 0.688$.
[b] Exact Fisher test $p = 0.688$.
[c] Mann–Whitney test, $p = 0.1708$.

appearance or progression of diseases. Therefore, the aim of this study is to perform histological and immunohistochemical analysis of the palatine and lingual tonsils of autopsied individuals with or without HIV infection.

## Materials and methods

### *Selection of individuals*

After approval by the Ethics Committee for Research in Human Beings of the Federal University of Triangulo Mineiro (UFTM), Protocol No. CEP-2547, a cross-sectional study was conducted with an analysis of complete autopsies performed at the Clinical Hospital/UFTM. All autopsies in which the tongue was removed from 1999 to 2011 were analyzed. Twenty-six individuals, half with HIV infection (HI) ($n = 13$) and half without HIV infection (CO) ($n = 13$), were selected. In this study, patients just were considered of the group HI if they actually had a western blot test positive for HIV and died from immune deficiency in combating AIDS-related co morbidities. No patient received antiretroviral therapy. Clinical records had no data on CD4 count and viral load because when the diagnosis of HIV infection was made, patients were already in the terminal stage, meaning there was not enough time for these exams. All patients with hepatitis or sepsis were excluded. In the CO group, we included patients who never had a positive test for HIV and showed no chronically infectious diseases (Table 1). Only two cases of infection in the HI group were acute infections. Data related to age, gender, and ethnicity (Caucasian and non-Caucasian) were collected. Cases in which the lingual and palatine tonsils were not removed during autopsies were excluded.

### *Removal of tonsil fragments, processing, and analysis of general pathological processes*

After forming groups, lingual tonsils fragments measuring 2 cm × 1 cm × 0.5 cm were removed from tongues previously kept in 3.7% formalin solution. The fragments were removed from the tongue with a scalpel blade in the form of a longitudinal segment. The palatine tonsils were completely removed and sectioned transversely for histological processing.

Next, the fragments were dehydrated in alcohol, diaphanized in xylene, embedded in paraffin, and processed for histopathological analysis. Six-micrometer tissue sections in the sagittal plane were cut and mounted on glass slides. The slides were stained with hematoxylin and eosin (HE) and picrosirius red. The slides stained with HE were used for morphometric analysis of follicles and to identify pathological processes such as inflammation, hemorrhage, and necrosis that were then classified as present or absent. The slides stained with picrosirius red were used for a morphometric collagen analysis.

### *Morphometric analysis of lymphoid follicles of both the palatine and lingual tonsils*

The morphometric analysis of the lymphoid follicles of palatine and lingual tonsils was performed. The stained slides used a 10x objective lens. An image analysis protocol was performed through the interactive method. For this morphometric evaluation, the slides were placed in a light microscope Axio 4.1 (Zeiss, Berlin, Germany) that was coupled to a color video camera AxioCam (Zeiss, Berlin, Germany) connected to a video capture card in a computer with the software program ImageJ (National Institutes of Health, Bethesda, USA). To determine the area occupied by each follicle, we outlined the contour of each follicle with a cursor. We measured all fields in which it was possible to observe well-organized lymphoid follicles. In each fragment we analyzed approximately four lymphoid follicles.

### *Morphometric analysis of collagen*

The morphometric collagen analysis was performed using an Axio 4.1light microscope (Zeiss, Berlin, Germany), an AxioCam (Zeiss, Berlin, Germany) camera capturing images, a computer with Axiovision 4.8 software (Zeiss, Berlin, Germany) with a 40× objective lens and a polarizing filter. The images shown in the microscope were transmitted to the computer monitor. In the polarized images, the collagen birefringence presented a reddish-yellow color and was automatically quantified. We analyzed all fields of the fragment, approximately 20 fields in all.

### *Immunohistochemistry*

#### *Immunohistochemistry protocol used for anti-CD31 and anti-tryptase*

For the immunohistochemical process for anti-CD31 and anti-tryptase, sections were deparaffinated, rehydrated, and washed with ultrapure water for 5 min at room temperature. The antigen retrieval was made using 0.01 M citric acid and pH 6 for 30 min. The slices were then incubated with 2% PBS/BSA (phosphate-buffered saline/bovine serum albumin) for 30 min to block non-specific binding.

The antibodies were diluted in 2% PBS/BSA in the following concentrations: anti-CD31 (1:50) (R&D, Minnesota, USA) and anti-tryptase (1:200) (R&D, Minnesota, USA). The sections were then incubated with diluted primary antibody for 18 h (overnight) at 4 °C. After this, the sections were washed twice with PBS and Tween 20 (0.05%). The slices were treated with 3% methanol and hydrogen peroxide (H2O2) for 15 min to block endogenous peroxidase of the tissues. For antibody detection, an avidin–biotin conjugated with peroxidase (ABC) technique was used with the LSAB-plus kit (DAKO, Carpinteria, USA). The complex was incubated for 30 min at room temperature and washed with PBS in the same manner as above.

Revelation was performed in 5–20 min using diaminobenzidine (DAB) (0.5 mg/ml) and 0.05% hydrogen peroxide at room temperature, protected from light. Subsequently, the sections were washed in distilled water, counterstained with Harris hematoxylin, and mounted with Entelan (Merck Millipore Brazil, São Paulo, Brazil).

#### *Immunohistochemistry analysis*

The slices immunostained for anti-CD31 and anti-tryptase were analyzed under an Eclipse light microscope (Nikon, Berlin, Germany) with a 40× objective lens. Mast cell and blood vessel counts were performed in all fields of the tonsillar stroma. To obtain the area evaluated, the area of each field evaluated on 40× objective was calculated with the use of a blade micrometer. The area of each field (0.14 $mm^2$) was multiplied by the number of fields evaluated