
Implications of systemic fungal infection in the atherosclerosis of autopsied patients

Implicações da infecção fúngica sistêmica no processo aterosclerótico de pacientes autopsiados

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Abstract

Objective – To describe the occurrence of systemic fungal infection in autopsied patients, to relate atherosclerosis to systemic fungal infection, and to quantify aortic collagen fibers of patients with systemic fungal infection. **Methods** – A retrospective study was carried out with a control group matched by age, and then patients with systemic fungal infection were identified and their aortas were analyzed both macroscopically and microscopically. **Results** – It was observed that 7% of patients had systemic fungal infection, of which 56% were infected by *Candida albicans*, 25% by *Cryptococcus* sp, 11% by *Pneumocystis jiroveci*, 4% by *Histoplasma capsulatum*, and 4% by *Paracoccidioides brasiliensis*. Macroscopic analysis of the group with systemic fungal infection showed that 66% had mild atherosclerosis, 17% had moderate atherosclerosis and 17% had severe atherosclerosis, whereas in the control group 47% had mild atherosclerosis, 43% had moderate atherosclerosis and 10% had severe atherosclerosis ($p > 0.05$). The amount of collagen in the group of patients with systemic fungal infection was significantly higher than in the control group ($p < 0.001$). **Conclusion** – The study suggests that in the macroscopic range of atherosclerosis there is no interference in systemic fungal infection; however, there was an increased production of collagen in the aortic wall of autopsied patients with systemic fungal infection, which may be due to the influence of the infection as well as to vessel inflammation, supporting the need to control and treatment.

Descriptors: Atherosclerosis/microbiology; Aortic diseases/microbiology; Autopsy; Fungi

Resumo

Objetivo – Descrever a ocorrência de infecção fúngica sistêmica em pacientes autopsiados, relacionar a aterosclerose e infecção fúngica sistêmica, e quantificar as fibras de colágeno da aorta de pacientes com infecção fúngica sistêmica. **Métodos** – Foi realizado um estudo retrospectivo com grupo controle pareado por idade, identificando-se os pacientes com infecção fúngica sistêmica, analisando as aortas de forma macroscópica e microscópica. **Resultados** – Verificou-se 7% de pacientes com infecção fúngica sistêmica; 56% por *Candida albicans*, 25% por *Cryptococcus* sp, 11% por *Pneumocystis jiroveci*, 4% por *Histoplasma capsulatum*, e 4% por *Paracoccidioides brasiliensis*. A análise macroscópica do grupo com infecção fúngica sistêmica mostrou que 66% apresentavam aterosclerose leve, 17% moderada e 17% grave. No grupo controle, 47% leve, 43% moderada e 10% grave ($p > 0,05$). A quantidade de colágeno no grupo de pacientes com infecção fúngica sistêmica foi significativamente maior do que no grupo controle de pacientes ($p < 0,001$). **Conclusão** – O estudo sugere que não há interferência na infecção sistêmica por fungos no âmbito macroscópico da aterosclerose, porém houve uma maior produção de colágeno na parede da aorta de pacientes autopsiados com infecção fúngica sistêmica, que pode ser devido à influência da infecção e inflamação dos vasos, mostrando necessário seu controle e tratamento.

Descritores: Aterosclerose/microbiologia; Doenças da aorta/microbiologia; Autopsia; Fungos

Introduction

Fungal infections have become increasingly prevalent due to the fact that some fungi live as saprophytes in normal individuals, and may therefore become pathogenic in immunosuppressed patients¹. Even though the presence of fungi in the human arteries is rare, it is of great importance since it is difficult to establish the diagnosis and without it there is a high probability of mortality among infected individuals. Pathological processes such as atherosclerosis and aneurysms can be triggered by opportunistic infections, for instance those caused by fungi². Atherosclerosis is a disease of the vessel wall that occurs due to innumerable factors and leads to atheromatous plaque formation. The increase in lipid deposits and deposits of immune system cells in the vessel wall reduces local blood flow and increases the deposition of connective tissue by fibroblasts. Therefore, the structure of the artery becomes rigid and

inflexible and calcium salts circulating in the blood may also precipitate in the atheromatous plaque and make the arteries stiff. Atherosclerosis can trigger blood clotting or even the rupture of a blood vessel. Factors such as high ingestion of saturated fats, sedentary lifestyle and genetic factors can be crucial for the development of this process³⁻⁴. Atherosclerosis is a worldwide disease which has complications and a high prevalence rate, besides being responsible for a high incidence of morbidity and mortality the world over⁵. It is considered to be the main cause of death in which common risk factors are not clearly identified, and it is often connected with environmental exposure. Experimental and clinical studies have showed that infection by microorganisms may accelerate the atherosclerotic process and other risk factors for this disease⁶. Hence, there is a great interaction between the infections, for instance fungi. The aorta is the largest artery in the

circulatory system and it is one of the most affected by fungi. Prevalence of infections caused by *Candida* sp and *Aspergillus* sp^{2,7-8} in the vessel wall is reported in the literature, but infections caused by *Cryptococcus* sp⁹ and *Trichoderma*¹⁰ may also be found.

Methods

The patients who had systemic fungal infection and some degree of atherosclerosis were identified through information gathering from the autopsy reports conducted at *Hospital de Clínicas* (General Hospital) of *Universidade Federal do Triângulo Mineiro*, in Uberaba, Minas Gerais state, Brazil, from 1994 to 2007. Patients aged 18 or older were selected, regardless of the cause of death or underlying disease. The aortas of patients of matching age and gender without fungal infection that were autopsied during the same period of time were regarded as control group. Later, macroscopic analysis of the aortas was carried out in order to determine the intensity of atherosclerosis. Quantitative analysis of this study led to the description of atherosclerosis as mild, moderate and severe. Extension of the atheromatous plaque, intensity of fibrosis, and calcification were taken into account in this evaluation. The aortas were analyzed by using a standardized scale ranging from 0.0 cm to 12.0 cm. Each examiner measured the degree of atherosclerosis in a subjective way using a non-millimetered scale, which registered one point. Then, measurement of the distance of 0.0 cm to the point marked on the scale was carried out with a scale ruler. Atherosclerosis was consensually classified regarding intensity in three grades: mild (from 0.1 to 4.0 cm), moderate (from 4.1 to 7.0 cm) and severe (from 7.1 to 12.0 cm). The method of morphological analysis of the aortas was adapted from other studies¹¹⁻¹². Aorta fragments of control group patients with systemic fungal infection were collected for the microscopic analysis, and were processed and stained by Picosirius method. In order to quantify collagen, the slides were divided into four quadrants and five measurements were performed in each of them. The image was captured using a polarized light microscope with a 20x objective and it was quantified

by the automatic image analysis software KS-300® (Kontron/Zeiss). The results were expressed as percentage (%) of the affected area. Macroscopic statistical analysis was carried out using chi-square test (X^2) so as to compare the systemic fungal infection group with the control group. The microscopic measurements, which showed abnormal distribution, were analyzed by Mann Whitney test. Spearman's correlation coefficient was used in order to correlate the amount of collagen to the age of patients with systemic fungal infection and of control group patients. The results were considered statistically significant when $p < 0.05$.

This study was approved by the Ethics Committee of Universidade Federal do Triângulo Mineiro, through the Protocol No: 1104/2008.

Results

Twenty-five cases of patients with systemic fungal infection who had some degree of atherosclerosis were discovered, corresponding to 7% of the autopsy reports. Occurrences of systemic fungal infection were as follows: 55% (n=15) were caused by *Candida albicans*, 30% (n=8) by *Cryptococcus neoformans*, 7% (n=2) by *Pneumocystis jiroveci*, 4% (n=1) by *Histoplasma capsulatum* and 4% (n=1) were caused by *Paracoccidioides brasiliensis*. The patients' ages ranged from 23 to 84 years, with an average age of 40.6 ± 16.2 years. Macroscopic analysis of the aortas of patients with systemic fungal infection showed that 66% of them had mild atherosclerosis, while 17% had moderate atherosclerosis and 17% had severe atherosclerosis. It was noticed that 47% of the patients in the control group had mild atherosclerosis and that 43% and 10% of them had moderate and severe atherosclerosis, respectively. Statistical analysis was not significant ($X^2 = 5.11$; $p > 0.05$). The amount of collagen in the group of patients with systemic fungal infection was significantly higher than the amount in the control group ($p < 0.001$) (Table 1). It was observed that collagen increased as patients with systemic fungal infection aged (Figure 1), while there was a decrease in collagen as the control group patients aged (Figure 2).

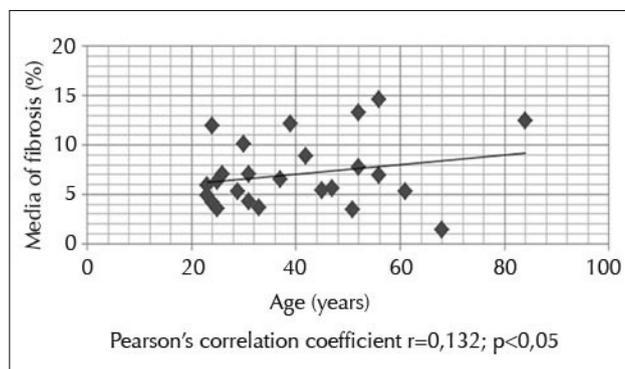


Figure 1. Correlation between the collagen percentage in the intima and media of aorta with age (patients with infection fungi)

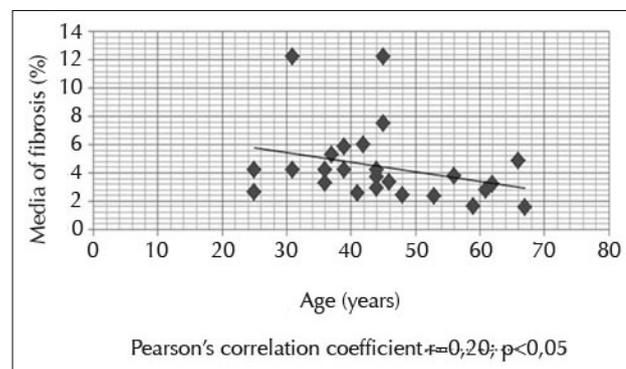


Figure 2. Correlation between the collagen percentage in the intima and media of aorta with age (patients of control group)

Table 1. Percentage of collagen in the intima and media of aorta (patients with systemic fungal infection and control group)

Group	Collagen in the intima and media of aorta (%)
Systemic fungal infection group patients	5,50 (0,06 - 40,71)
Control group patients	3,27 (0,09 - 24,68)

T= 288981,500 ; p<0,001

Discussion

Epidemiologic study of patients who had atherosclerosis and systemic fungal infection allowed us to analyze the distribution of infectious agents and showed the prevalence of each of them in the group of patients studied. This epidemiologic study is of extreme importance given that systemic fungal infection may lead to sepsis and even to a septic shock. The diagnosis of systemic fungal infection is essential due to the increase in this type of infection on the microbiological level¹³. The present study showed that no interference in the macroscopic level of the systemic fungal infection of atherosclerosis was found in the autopsied patients. However, it does not mean that such interference has not happened in other cases since there are reports in the literature suggesting that opportunistic infections, such as fungal infections, cause atherosclerosis². This study aims to contribute by alerting people to the fact that there are cases in which fungal infections may lead to atherosclerosis, although such cases are rare². Therefore, control of fungal infection is required in order to control atherosclerosis as well. Furthermore, this study showed a higher amount of collagen in the arteries of patients with fungal infection compared to the amount found in the arteries of patients without the infection. This may be due to pro-inflammatory processes (through IL-1, INF- γ and TNF- α) triggered by the systemic fungal infection. It is known that these mediators, present in the physiopathogeny of fungal infection, may lead to a vessel wall lesion with subsequent fibrosis. The decrease in collagen content as control group patients aged is not in accordance with the literature¹⁴⁻¹⁵. However, this might be due to other diseases these patients had which could have interfered in the fibrotic process.

Conclusion

Thus, the study suggests that in the macroscopic range of atherosclerosis there is no interference in systemic fungal infection, however, there was an increased production of collagen in the aortic wall of autopsied patients with systemic fungal infection, which may be due to the influence of the infection as well as to vessel inflammation, supporting the need to control and treatment.

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