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Camparison of Oral Candida Flora in Patients with Coronary Atherosclerosis and Healthy People

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Abstract

***Background*:** Coronary atherosclerosis is a common disorder of the arteries and may block the arteries and cause heart disease. Recently several studies have indicated a role of infectious agents in atherosclerosis and obstructive coronary artery disease. Candida species are normal flora of the human oral cavity and can enter in the blood stream and damage inner walls of coronary arteries by several mechanisms. Thus this study was done to compare the oral candida flora in healthy people and patient with coronary atherosclerosis.

***Materials and Methods*:** In this study, we compared oral candida flora in 90 patients with coronary atherosclerosis and 90 healthy people. All specimens were obtained from oral cavity by swab and cultured on CHOROMagar Candida medium. Identification of isolated colonies was done by RapID yeast plus system. The data were analyzed using the chi-square test.

***Results*:** In this study 55.4% (N=61) of patient and 44.6% (N=49) of control group had candida colonization in their oral cavity. *Candida albicans* was the most common species isolated in both groups. *Candida glabrata*, *Candida krusei*, *Candida tropicalis* and *Candida parapsilosis* were the most common non albicans species. Although no significant difference was observed between candida colonization in patients and controls, the differences between isolated colony numbers in patient and healthy group were significant (p=0.001).

***Conclusion*:** Hypercolonization of candida species in oral cavity may lead to invasion and enter the organism in the blood stream and damage the coronary arteries.

Introduction

Atherosclerosis is a public health challenge in industrial countries. It accounts for 42% of mortality in the United States, and some half of these cases pertain to coronary artery atherosclerosis [1]. The classic risk factors include hypertension, hyperlipidemia, smoking, diet and family history [2, 3]. Nevertheless, more than 30% of individuals who expire as a result of coronary atherosclerosis lack any of these classic risk factors [4]. It has recently been demonstrated that microbial agents are involved in pathogenesis of atherosclerosis [5]. Several infectious agents, including *Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus, *Herpes simplex* virus, and *Porphyromonas gingivalis*, have been incriminated in the development of atherosclerosis [6]. Candida species constitute the normal flora of the oral cavity in 30-50% of individuals and may cause infection under certain conditions. Moreover, they can enter the bloodstream using their hydrolytic enzymes and transfer to other locations in the body [7]. Candida species may be involved in vascular lesions with platelet aggregation and endothelial adhesion [8]. Therefore, they are considered among microbial agents involved in coronary artery disease. As few studies have been conducted to address this issue, the present study aims to investigate and compare the type and degree of candida colonization in the oral cavity of patients with coronary atherosclerosis to that of health individuals.

Materials and Methods

This is a case-control study on individuals referring to the heart clinic of Imam Khomeini Hospital, Tehran University of Medical Sciences for diagnostic angiography. Once angiography was completed by a cardiologist, 90 individuals with at least 50% stenosis in coronary artery were selected as the case group, and 90 individuals without stenosis or with less than 50%

stenosis in their coronary arteries were selected as the control group. A questionnaire was developed to inquire about the individuals' conditions in terms of family history of heart disease, diabetes, smoking, hypertension, hyperlipidemia, tooth decay, and use of antifungal and antibacterial antibiotics in the last two months. Samples were obtained from the oral cavity using a wet swab. The swab was shaken well in sterile physiology serum to have the yeasts separate from it and enter the serum. The resulting suspension was then centrifuged and 50 mL of the sediment was cultured on CHROMagar Candida medium. Part of the sediment was used to prepare wet smear with 10 KOH. The resulting smears were studied microscopically for yeasts and micelle components of candida. The CHROMagar medium was preserved at 35ºC for 48 hours. Subsequently, the colonies isolated on CHROMagar were sampled and cultured on cornmeal agar with tween 80 at 25ºC for 4 days to distinguish albicans and non-albicans species. We then prepared lactophenol cotton blue smears from colonies cultured on this medium, and studied the slides under microscope. *Candida albicans* creates chlamydospores on this medium, whereas non-albicans species fail to do so [9]. We determined the species of non-albicans species using Rapid Yeast Plus System following the instructions of the kit, and analyzed the data using chi-square test.

Results

In this study, 90 patients with coronary artery stenosis and 90 individuals in the control group were compared. The two groups were not significantly different in terms of their background diseases (Table 1). Sixty one patients (67.7%) in the case group and 49 individuals (54.4%) in the control group had candida colonization in their oral cavity, yielding no statistically significant difference between the two groups (χ^2=3.366; p>0.05; OR=1.76; CI=0.96-3.22) (Table 2). However, considering the total number of candida colonies isolated from the case and control groups (28,672 versus 18,469, respectively), we observed a significant difference in the number and intensity of candida colonization between the two groups (χ^2=2208; p=0.001). The most common species isolated from patients and controls was *C. albicans*, and the most common non-albicans species was *Candida glabrata* in patients and control; *Candida kefyr* was isolated from controls only (Table 3).

Discussion

In the present study, the status of candida colonization was not significantly different between cases and controls, which are probably due to the small sample size of the study; nevertheless, the total colony count from patients was significantly greater than that of the control group, thus posing the possibility of a relationship between candida species and coronary atherosclerosis. We isolated five candida species in our study: *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. kefyr*, and *C. krusei*.

Infectious agents probably exert their effect on atherosclerosis through endothelial injury and stimulating the autoimmune response to heat shock proteins [10].

Considering the results of previous studies, it may be stated that there is little data on the role of fungal species in development of atherosclerosis and most studies have dealt with bacteria or viruses. Miyashita et al. studied 160 patients with cardiovascular diseases for antibody against *Chlamydia pneumoniae* and compared the results with 160 healthy individuals; they reported a significant relationship between cardiovascular diseases and *Chlamydia pneumoniae* infection [11].

In another study, Roivainen et al. evaluated the antibody level against herpes simplex and *Chlamydia pneumoniae* in 241 patients with cardiovascular disease and 241 healthy individuals to observe a significant relationship only between *Herpes simplex* infection and cardiovascular disease [12].

Ott et al. studied 38 patients with atherosclerosis and found fungal 18s rDNA in 92% of atherosclerotic plaques [13]. Candida species occur in the oral cavity of 30-50% of individuals and their role in development of certain cardiovascular diseases, such as endocarditis and pericarditis, is well established [14, 15].

Table 1. Comparing the absolute and relative frequency of risk factors for coronary atherosclerosis in patients and controls

Group / Risk Factor	Patients N(%)	Healthy N(%)	*p*-Value
Family history of heart disease	25(27.7)	23(25.5)	0.88
Diabetes	21(23.3)	22(24.4)	0.86
Smoking	19(21.1)	17(18.8)	0.7
Hypertension	32(35.5)	29(32.2)	0.63
Hyperlipidemia	27(30)	25(27.2)	0.74
Tooth decay	24(26.6)	27(30)	062

Table 2. Comparing the absolute and relative frequency of candida colonization in patients and controls

Group / Colonization Status	Patients N(%)	Healthy N(%)	*p*-Value
Positive	61(67.7)	49(54.4)	
Negative	29(32.3)	41(45.6)	0.067
Total	90(100)	90(100)	

Table 3. Colony count and candida species isolated from oral cavity of patients and controls

Candida species / Group	Albicans	Tropicalis	Glabrata	Kefyr	Krusei	Total
Case	13548	2977	9582	0	2565	28672
Control	6613	1491	5557	32	4776	18469
p-Values	0.001	0.03	0.018	0.045	0.024	0.001

To the best of our knowledge, this is the first study to address candida species in the oral mucosa of patients with coronary atherosclerosis. Miura et al. demonstrated that in DBA/2 rats, *C. albicans* causes coronary artery inflammation, increased heart weight, and death. Candida induces the secretion of CSF GM which plays an essential role in atherosclerosis and acute coronary syndrome. This cytokine stimulates the release of gamma interferon, as well, which is involved in atherosclerosis. This increased production of this cytokine may be one of the mechanisms through which candida species favor cardiovascular diseases [16]. Candida species may adhere to endothelial surfaces, with the greatest adhesive ability pertaining to *C. albicans*, *C. tropicalis* and *C. krusei*, and the smallest ability belonging to *C. parapsylosis*, *C. pseudotropicalis*, and *C. glabrata* [17].

In the present study, we found a significant difference between the two groups in terms of colony counts for *C. albicans*, *C. tropicalis* and *C. krusei*. As these species are particularly potent in adhering to vascular endothelia, it is presumable that they should be involved in endothelial injury and development of coronary diseases. Therefore, it is necessary to control the candida flora in oral cavity, especially in patients with coronary atherosclerosis. In our study, *C. albicans* was the most frequent species. Candida species pass through the endothelium with their aspartyl proteinase and enter the bloodstream. *C. albicans* has the highest amount of aspartyl proteinase gene, and thus is better equipped to penetrate endothelia [7, 18]. In bloodstream, *C. albicans* secretes hemolysin and releases hemoglobin from red cells. Hemoglobin contributes to the expression of genes involved in production of germ tube by *C. albicans*, and the germ tube plays a crucial role in pathogenicity of *C. albicans* [19]. In addition, hemoglobin stimulates the expression of fibronectin receptors in *C. albicans*, thus improving its adhesion [20].

This enhanced adhesion may contribute to the formation of atherosclerotic plaques in vessels. Some cytokines secreted by leukocytes injure endothelial cells. Tumor necrosis factor alpha (TNF-α) and interleukin-6 are the most important cytokines linked to atherosclerosis and *C. albicans* stimulates their secretion. TNF-α activates inflammatory cells and produces metalloproteinase to detach the atherosclerotic plaque, mobilize it in bloodstream and occlude smaller vessels, thus raising the risk of heart attack. Interleukin-6 stimulates the production of C-reactive protein (CRP) in the liver and thus contributes to cardiovascular diseases [16, 21]. Therefore, the albicans species may assume a crucial role in pathogenesis of coronary atherosclerosis. Candida species also enhance their adhesion to endothelial walls through stimulating platelet aggregation, which may raise the risk of cardiovascular diseases [22]. In the present study, we did not find a significant relationship between intensity of candida colonization and severity of atherogenesis, hypertension, hyperlipidemia, tooth decay and diabetes. Nonetheless, our findings pose the possibility of a role for candida species in pathogenesis of coronary atherosclerosis through increased oral colonization of candida. Our study has two limitations: 1- the small number of patients and poor compliance in some cases; 2- more patients undergoing angiography had more than 50% stenosis, thus leaving only a few individuals for the control group. Hypercolonization of candida species in the oral cavity of patients with coronary atherosclerosis may be a prelude to candida invasion and entry into bloodstream. Considering the ability of candida in causing vascular endothelial injury and platelet aggregation, this fungal organism may be an etiologic factor for occlusive lesions in coronary arteries.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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