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The Relationship between Candida Infections and Atherosclerotic Heart Disease

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ABSTRACT:

Background: The purpose of this investigation is to determine whether or not fungus belonging to the genus Candida can be found within atherosclerotic plaques and to examine various immunological and biochemical indices in patients with particular cardiac conditions who also harbour Candida spp. Specifically, here's what to do and how to do it: In order to determine if fungi were present in an atherosclerotic plaque, a specific test was performed.

Method: Twenty autopsies revealed a total of 61 atherosclerotic plaques. In addition, 41 persons with atherosclerosis had their blood tested for TNF-α and IgG and IgM antibodies to Candida spp. There were a wide range of ages represented in this group, from 34 to 85 years old, with 28 men and 13 women.

Results: The results showed that fungal species belonging to the genus Candida were present in 24.9% (10/41) of atherosclerotic plaques. *Candida krusei* and *C. dubliniensis* were the most common types of Candida identified, while occasionally C. tropicalis and C. albicans were discovered. None of the 41 patients tested positive for IgM, although 30 of them (73.1% of the total) did test positive for IgG. There were fewer IgG-negative patients (n=6) with TNF-α than IgG-positive patients (n=11), although the TNF-α levels in the former were significantly greater than those in the latter (511.7pg/ml, p <0.05). It was shown that the discrepancies between ASAT and ALT levels were much larger in patients who tested positive for Candida spp but negative for TNF-α.

Conclusion: Candida fungus may contribute to atherosclerosis by inducing inflammation in the lining of blood vessels.

KEYWORDS: Candida albicans – Candida infection – fungal infections – heart disease

INTRODUCTION

There is more than one root cause of heart disease and, more especially, atherosclerosis. This concept, known as the etiopathogenesis of atherosclerosis, suggests that several forms of atherosclerosis can cause harm to blood vessels and, ultimately, early incapacity and death. The inflammatory theory, which suggests that many infectious agents may contribute to the development of atherosclerosis, gained a lot of traction toward the end of the twentieth century [1,2]. Epidemiological studies, serological examinations, the detection of bacterial and viral antigens, the isolation of pure cultures of specific microorganisms from the affected organ [3], and the inoculation of experimental animals are just a few of the methods that can be used to determine whether or not a somatic disease has an infectious aetiology [4]. Along with serological testing, fluorescence microscopy is frequently used to examine the contents of atherosclerotic plaques. Chlamydia pneumoniae, Cytomegalovirus, and Herpes simplex were all searched for using this technique in atherosclerotic plaques. Infectious agents like Chlamydia pneumoniae, Cytomegalovirus, Herpes simplex, Mycoplasma infection, and Helicobacter pilori are now known to contribute to the development of cardiovascular disease [5]. A few characteristics set these infectious agents apart from others are their capacity to exploit their host cells' resources, their ability to dampen the immune system, and their persistence within an organism [6]. However, the genus Candida contains fungus with characteristics like those described above. Candida species fungi, which are ubiquitous in the human body, can cause candidiasis in those with compromised immune systems [7].

Candida consists of two main types of fungi, *Candida albicans* and non-*Candida albicans Candida* (NCAC). Candida species are commonly found on the surface of the skin and mucous membranes, where they remain for the duration of the host's life. *C. albicans* is the only species of Candida that is known to cause disease in humans, while other species of NCAC such as *C. auris, C. glabrata, C. parapsilosis, C. tropicalis, C. guilliermondii, C. krusei, and C. dubliniensis* are also possible [8].

However, even among *Candida* species, there is a wide range of diversity in terms of morphogenesis, pathogenicity, and other phenotypic traits. There may be a correlation between various *Candida* species and particular patients, as suggested by a few authors. Case in point: HIV-positive individuals who suffer from oral thrush may also be infected with *Candida dubliniensis*. Patient populations at increased risk for *C. parapsilosis* include those who have undergone transcatheter aortic valve replacement (TAVR), are receiving a transplant, or are receiving parenteral nourishment (PN). Blood cultures typically contain *C. parapsilosis* as the second most prevalent species. The yeast Candida albicans is the most prevalent type [9]. *Candida albicans*, the most prevalent kind of fungus, has been shown to produce compounds, such as adhesions and ligands, that prevent neutrophils from adhering to it and leukocytes from migrating toward it [10]. Candida fungi rely heavily on "external antigens," which are chemicals that help them colonise, adhere, and infect hosts. Multiple analyses have demonstrated that a *Candida* culture can produce a a water-soluble high molecular weight fraction (CAWS) that is both water-soluble and contains mannoproteins and -glycan. Research on the biological effects of CAWS has shown a few interesting findings [11]. CAWS stimulates IFN- and IL-6 production in splenocytes, even at a modest dose (10 g/ml). High concentrations of CAWS inhibit splenocyte proliferation induced by polysaccharides and T-cell mitogens. Additionally, CAWS inhibits endothelial cell trombodulin production and collaborates with TNF to activate the blood's halting system [12]. Artheritis, cardiomegaly, and increased levels of pro-inflammatory cytokines have all been observed in mice fed 4 mg of a highly filtered solution of CAWS in laboratory experiments. That being said, the vital activity of the fungus Candida in an organism affects the blood vessels both directly and by the cytokines that are created in reaction to the damage [13].

PATIENTS AND METHODS

Our research included 41 CHD patients admitted to hospitals in 2021 and 2022). All of the patients, according to the angiograms, had severely narrowed arteries (greater than 50% stenosis in at least three coronary arteries). There was just one option for treatment, and that was a bypass of the coronary arteries (CABG). Factors unique to each patient were considered, including but not limited to age, sex, smoking status, body mass index, lipid profile, blood pressure, cholesterol levels, and family history of cardiovascular disease and diabetes.

All patients who participated in the study also signed a consent form indicating that they had been informed of the nature of the investigation and gave their informed consent.

In order to identify *Candida albicans* in the atherosclerotic plaque composition, we applied the cultural diagnostics approach. To learn more about the role *Candida albicans* plays in atherosclerosis, we developed and implemented a novel approach to detecting microorganisms in an atherosclerotic plaque ("Method for identifying a likely cause of atherosclerosis"). Our technique relies on cultivating microorganisms from a biological substance to identify their species.

The goal of a cultural diagnosis is to isolate the bacterium in question from the sample of tissue or other biological material. By isolating a pure culture of the microbe and analysing its biological properties, the general and specific attribution of the causal agent can be made. Scientific samples are collected from the object of study and disseminated across various culture media on a sterilised dish. The temperature in an incubator set for cultivating microorganisms is maintained at 36 degrees Celsius. Any growth that is taking place will be evident after 5 days. A variety of factors, including microscopic observations of diseased tissue, culture features, biochemical activity, etc., are used to pinpoint the identity of the offending microbe.

Our task was to determine, using culture diagnostics, what kinds of bacteria were located in the atherosclerotic plaque core and base.

Parts of the descending aorta measuring 3–5 centimetres by 3–5 centimetres with at least 2 intact athromatous plaques measuring at least 0.5 centimetres in size were chosen for the bacteriological analysis. If it had been less than 24 hours since the person's death and there were no evidence of chronic infectious diseases, oncological disorders, or abscesses, then the aorta would be removed during an autopsy. This held true regardless of the manner of death. Tissue from the aorta was transferred to the lab in a sterile dish after being washed by hand to remove blood clots and dried on a clean rug (a disposable plastic cup with a Petri lid). The inoculation with bacteria was performed in a laboratory setting, and each step was documented with digital photographs. Unaltered plaques of at least 0.5 cm in size were examined, indicating the presence of atheromatous bulk. The operculum of the plaque is punctured if its diameter is larger than roughly 0.5 cm.

After preparing the injection site with alcohol, a sterile puncture needle is injected into the plaque on the lumens side, cut side up, parallel to the aorta. Alcohol is used to open up large plaques or calcium plaques before they are sliced up. After cleaning the wire inoculating loop, why do we need to use tweezers, scissors, and a needle to make the inoculation? Selecting a plaque at least 0.5 cm in size, the operculum is removed by gripping it with tweezers on both sides and heating it over a flame. Using the wire inoculating loop, carefully remove a piece of the mass without contacting the sides if there is atheromatous tissue inside. Then, the mush should be dispersed on Sabouraud's medium, blood agar-agar, sugar broth, and culture medium

No. 2 GRM (ready medium for fungus identification). A mass was re-seeded on HiCrome Candida Agar to determine what kind of fungus it was (HiCrome Candida differential Agar). Candida chromogenic agar is a differential and selective medium for the rapid isolation of the fungus from complex culture mixtures, as well as the colour and morphological distinction of *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and Can-didagrabrata fungal colonies. If there isn't enough atheromatous mass to scrape off the top, the contents of the plaque are placed on the designated culture media. Once infected, the culture medium spends the next seven days in an incubator set to 30 to 32 degrees Celsius. Small colonies should begin to appear on the medium on day two. These colonies are white and prominent on Sabouraud's medium, however the colour of the colony on HiCrome Candida Agar varies with the kind of fungi.

When using HiCrome Candida Agar, the proposed method for identifying microorganisms in atheromatous plaques is effective enough because it can screen out Candida-type fungus. Candida-type fungus may be isolated from atheromatous plaques roughly 30% of the time.

The atherosclerotic plaques that were removed during surgery were divided into three sections. Separate sterile tubes containing normal saline and formalin were used to store each specimen for later use in culture and pathology. For molecular analysis, samples were transported to the lab in a cold box and placed in tubes that had been sterilised with 70% alcohol to kill any remaining DNA or RNA.

A number of different media were used to cultivate the specimens, including CHRO-Magar Candida agar, Sabouraud dextrose agar, Sabouraud dextrose agar with chloramphenicol, Brain heart infusion agar, Blood agar, and Eosin methylene blue agar. Direct microscopical evaluation of clinical specimens for fungal components using a solution containing 10% KOH was performed. For a thorough pathological analysis, we stained tissue sections with Gomorimethenamine silver (GMS), hematoxylin and eosin (H&E), and periodic acid Schiff (PAS).

The demographic data was analysed using the Chi Square test in SPSS (2022, USA) version 15, and a P value of 0.05 was considered to be statistically significant.

RESULTS

None of the samples tested positive for the presence of fungi when examined directly with 10% KOH, but one did when cultivated in a culture; this one developed into a colony of mould. Conidia were unicellular, spherical or cylindrical, and hyaline, while the mycelium was hyaline, septate, and branching in a slide culture.

The presence of microorganisms in atherosclerotic plaques provides further evidence linking them to the disease. Fluorescent microscopy is currently the method of choice for this. However, this technique calls for pricey equipment and chemicals, and the expertise of the technician doing the treatment is crucial.

Fungi belonging to the genus Candida were discovered in the contents of 24.9% of atherosclerotic plaques. To find that the most common species were *C. krusei*and *C. dubliniensis,* but a single colony of *Candida albicans* was also found.

As shown in the following figure,

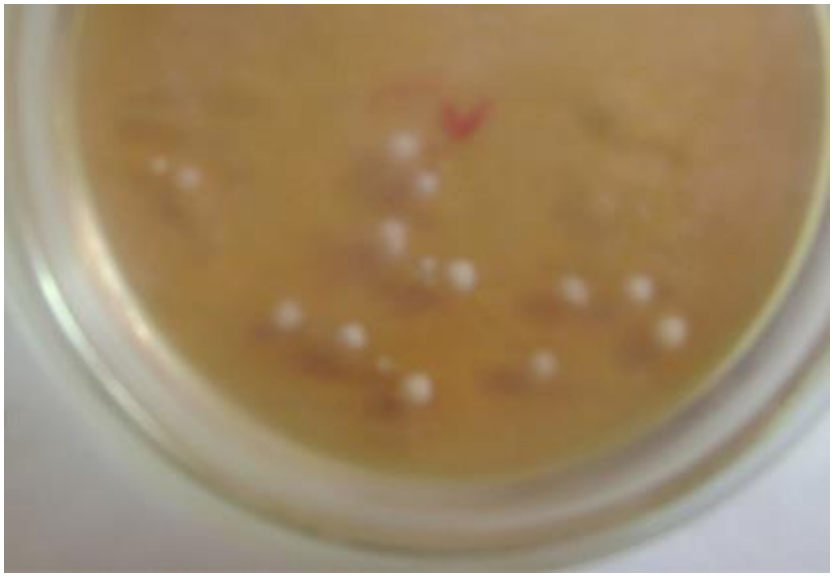

Figure 1: *Candida* spp. in Saburo medium from atherosclerotic plaque (7th day)

The Relationship between Candida Infections and Atherosclerotic Heart Disease

First and foremost, the existence of antibodies against the causal agent indicates illness, and to some extent, the degree to which the body has been sensitized to the infection. Eighty individuals were tested using ELISA, and the results showed that not a single one of them had IgM-class antibodies.

Out of 41 patients, 30 (73.2% of the total) were negative for IgG class antibodies to *Candida albicans*.

IgG *Candida albicans* positivity was used to classify individuals into one of two categories (group I: negative, group II: positive). According to the inflammatory theory of atherogenesis, the instability of the plaque is linked to inflammation, and inflammation is clinically exhibited in the form of an acute coronary syndrome.

Group I (30 patients) was found to have an average TNF- level of 511.73 195.80 pg/ml, with 11 patients (26.8%) having detectable levels. TNF- was found in 6 of the 11 patients (54.5% detection rate) in group II, with an average level of 326.70 pg/ml. TNF- frequency was found to vary with the presence or absence of IgG antibodies against *Candida albicans*, as determined by the chi-squared test for nonparametric agreement. Comparing the two groups, there is a statistically significant difference in TNF- levels (Mann-Yitny test, $p< 0.05$). The following figure shows the distribution of TNF among the two groups.

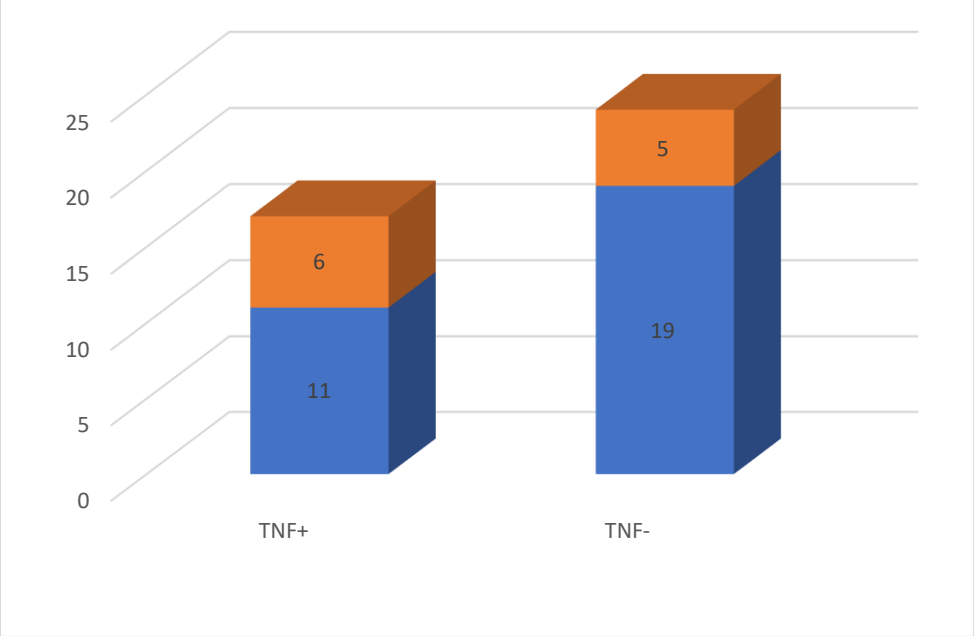

Figure 2 Percentage of TNF in the plaques

Statistics from blood tests for patients who tested positive and negative for Candida, broken down by the presence or absence of TNF-, are presented in the table below,

	Group 1		Group 2		Significance level
	TNF-α positive	TNF-α negative	TNF-α positive	TNF-α negative	
n	**11**	**19**	**6**	**5**	
AST	11.2 ± 1.4	14.1 ± 1.1	21.9 ± 1.4	13.4 ± 0.9	
ALT	14.6 ± 2.2	17.4 ± 1.4	19.2 ± 2.3	16.8 ± 1.7	
Total bilirubin	16.8 ± 1.3	17.1 ± 2.2	14.3 ± 2.1	19.1 ± 1.1	
Conjugated bilirubin	5.5 ± 0.9	4.8 ± 1.5	4.3 ± 3.2	5.1 ± 0.7	
Total protein	68.7 ± 1.2	70.1 ± 1.2	71.2 ± 0.7	69.4 ± 5.6	
Total cholesterol	5.2 ± 3.1	5.9 ± 1	5.3 ± 1.2	5.1 ± 0.1	
High density lipoprotein (HDL)	1.1 ± 1.3	1.2 ± 0.9	1.2 ± 1.3	1.1 ± 0.9	
Low density lipoprotein (LDL)	3.2 ± 1.1	3.3 ± 2.1	3.5 ± 2.4	3.6 ± 1.1	
Urea	6.5 ± 0.5	6.9 ± 3.1	6.3 ± 0.4	6.2 ± 2.1	$p< 0.05$
Triglycerides	1.4 ± 1.7	1.7 ± 1.4	1.5 ± 1.4	1.9 ± 1	
ALP	79 ± 0.5	91 ± 0.8	87 ± 2.3	94 ± 2.2	

DISCUSSION

Over the past several years, there has been a rise in the amount of studies that demonstrates the role pathogenic microorganisms play in the advancement of atherosclerosis. Fungi have only been the subject of a very small number of research, in contrast to the many that have been conducted on viral and bacterial infections. Direct examination, culture, histology, and PCR-sequencing were used to investigate the function of fungi in the progression of atherosclerosis in patients with angiographically confirmed coronary heart disease greater than 50% stenosis in at least three coronary arteries [14,15].

These patients had coronary artery disease that had been confirmed by angiogram. Even though our research found evidence of fungal development in 26.8% of atherosclerotic plaques, we were unable to establish any statistical evidence that connects fungal growth to atherosclerosis. In addition, the presence of fungal components in atherosclerotic plaques was not shown to be related to age, sex, smoking, obesity, high blood pressure, high cholesterol, a family history of heart disease, or diabetes [16]. These factors were not found to be associated with the presence of fungal components. Fungi were identified in 92.11 percent (35 of 38) of the atherosclerotic plaque samples that Ott and his colleagues examined. As a direct consequence of this, fungi were thought to be a general cause that underpinned atherosclerosis. The findings of this study are in contrast to the findings of the study that was discussed earlier. Differences in findings between studies could be attributed to a number of factors, including the different patient populations, study settings, and diagnostic criteria used. However, we found that 31.9% of atherosclerotic plaques (15/47) included fungi of the species *Candida*, which is in line with the research that Maya J. Nurgeldiyeva and her colleagues conducted [17].

Candida species are common among the fungus that can be found in the oral and intestinal tract flora. It is probable that oral and gastrointestinal bacteria prevail in atherosclerotic plaques. Yeasts have the potential to enter the bloodstream via cuts that occur when brushing one's teeth as well as through more invasive medical procedures such as having a tooth extracted, undergoing root canal therapy, or undergoing surgery [18]. This disease is referred to as a transient fungalemia. Platelet aggregation is what happens when platelets gather together to cover fungus in the bloodstream. This process has a name: platelet aggregation. Due to the fact that fungi are able to cling to the endothelium that lines the blood vessels, this increases the risk of thrombosis as well as cardiovascular disease. Taheri *et al*. [19] investigated the oral Candida flora of 90 individuals who had coronary atherosclerosis and 90 individuals who did not have the condition. It was found that there was no significant difference in either the number of Candida colonies or the total amount of Candida colonisation between the patients and the controls [20].

For the purpose of our study, we restricted our attention to only those patients whose coronary heart disease could be gleaned through an angiogram. Because we used a wide variety of diagnostic methods to search for fungi in atherosclerotic plaques, the findings of our research are more trustworthy than those of other studies, which is an extra plus. However, the study may have some shortcomings due to the fact that it used such a limited number of participants.

When we tested for IgG class antibodies against *Candida albicans* and tumour necrosis factor, we did not find a significant correlation between the presence of these antibodies and changes in blood lipids. This was the case even though we found a correlation between the presence of these antibodies and changes in blood lipids [21].

According to the findings of this study, the Candida fungus may cause inflammation of the artery wall, which in turn may lead to the development of atherosclerosis. However, the findings of our research do not present any evidence that can be taken as proof that fungi are the principal agents responsible for the degradation of vascular walls. This is because fungi are able to colonise and grow inside of phagocytes, and phagocytes are then able to deliver the fungi to the plaque when they migrate into the intima of the channel. This is the reason why this occurs.

It has been discovered that in artificially manufactured habitats, *Candida krusii* and *Candida grabrata* are more common than *Candida albicans*, which is considered to be more of an anomaly. This is in line with the pattern that has been observed, which shows that the amount of candidiasis produced by *Candida albicans* has been going down, while the amount caused by Candida krusii and Candida grabrata has been going up. There is a possibility that the antifungal medication fluconazole, which belongs to the three azol family, is to fault. Fluconazole is only effective against *Candida albicans*; the fungus *C. krusei,C. dubliniensis,Candida krusei,* and *Candida grabrata* are resistant to it by nature. Fluconazole is very powerful against *Candida albicans* [22].

CONCLUSION

Candida infections are common and cause serious heart diseases like atherosclerosis, despite the fact that there is a strong link between the presence of candida fungi and atherogenesis and atherosclerotic disease and despite the fact that there have been many technical ways to prevent candida infections over the years. The findings provide useful information on how to

avoid fungal infections and do not rule out their possible impact in atherosclerosis and coronary artery disease. More research with a larger sample size are needed to determine the impact of fungus on atherosclerosis and coronary heart disease.

Conflict of interests: The author declare no conflict of interests.

REFERENCES

1) Sastry PS. Occult fungal infection is the underlying patogenic cause of atherogenesis. Med Hypotheses. 2004;63:671–674. [PubMed] [Google Scholar]
2) Akberov RF, Shafareyev AZ, Mihaylov MH. Progressing multifocal atherosclerosis: an etiology, clinical-radio diagnostics, modern aspects of treatment. Kazan: Ideal-Press publishing; 2008. [Google Scholar]
3) Kiyoshi K, Shingo Y, Miura NN, Horie S, Usui Y, Adachi Y, Yadomae T, Ohno N. Effect of CAWS, a mannoprotein-β-glican Complex of *Candida albicans*, on leukocyte, Endotelial Cell and Platelet Functions *in vitro* . Biol Pharm Bull. 2003;26:233–240. [PubMed] [Google Scholar]
4) Leroy O, Gangneux JP, Montravers P, Mira JP, Gouin F, Sollet JP, Carlet J, Reynes J, Rosenheim M, Regnier B, Lortholary O AmarCand Study Group. Epidemiology, Management and Risk Factor for Death of Invasive *Candida* Infections in Critical Care: A Multicenter, Prospective, Observational Study in Franse. Crit Care Med. 2009;37:1612–1618. [PubMed] [Google Scholar]
5) Wang CC, Lin SK, Tseng YF, Hsu CS, Tseng TC, Lin HH, Wang LY, Kao JH. Elevator of serum aminotransferase activity increases risk of carotid atherosclerosis in patients with non-alcoholic fatty liver disease. J GastroenterolHepatol. 2009;24:1411–1416. [PubMed] [Google Scholar]
6) Shiu B, Viira Y, Taker V, Fong I. Chlamidiapneumaniae (ChP), cytomegalovirus (C) and virus Herpes simplex (VHS) in an atherosclerosis of the carotid artery. Int Med Journal. 1998;2:136–138. [Google Scholar]
7) Vatutin NT, Chupina VA. Infection as the factor of development of the atherosclerosis and its complications. Cardiology. 2000;2:67–71. [Google Scholar]
8) Nurgeldiyeva MJ, Hojakuliyev BG, Muhammedov MB. Correlation of atherogenesis with an infection of Candida albicans. Int J ClinExp Med. 2014;7(8):2137-43. PMID: 25232398; PMCID: PMC4161558.
9) Muhlestein JB, Hammond EH, Carlquist JF, Radicke E, Thomson MJ, Karagounis LA, Woods ML, Anderson JL. Increased Indence of *chlamydia* species within the coronary arteries of patents with symptomatic atherosclerotic versus other forms of cardiovascular disease. J Am CollCardiol. 1996;7:1555–1561. [PubMed] [Google Scholar]
10) Mamtani S, Aljanabi NM, Gupta Rauniyar RP, Acharya A, Malik BH. Candida Endocarditis: A Review of the Pathogenesis, Morphology, Risk Factors, and Management of an Emerging and Serious Condition. Cureus. 2020;12(1):e6695. doi: 10.7759/cureus.6695. PMID: 32104631; PMCID: PMC7026878.
11) Nagi-Miura N, Harada T, Shinohara H, Kurihara K, Adachi Y, Ishida-Okawara A, Oharaseki T, Takahashi K, Naoe S, Suzuki K, Ohno N. Lethal and severe coronary arteritis in DBA/2 mice induced by fungal pathogen, CAWS, *Candida albicans* water-soluble fraction. Atherosclerosis. 2006;186:310–320. [PubMed] [Google Scholar]
12) Szkaradkiewich A, Bogaczy E, Ska-Staniewicz J, Torliński L, Wal M. Cytomegalovirus infection and atherosclerosis. Med SciMonit. 2002;8:104–7. [Google Scholar]
13) Masoumi O, Shahzadi M, Kordbacheh P, Zaini F, Mahmoudi S, Mahmoudi M, Bahreini H, Safara M, Mirhendi H. Detection of Fungal Elements in Atherosclerotic Plaques Using Mycological, Pathological and Molecular Methods. Iran J Public Health. 2015 Aug;44(8):1121-5. PMID: 26587476; PMCID: PMC4645732.
14) Yulish YI, Nagornaya NV. Chronic endocellular infections and cardiovascular pathology. Clinical Lectures. 2007:2. [Google Scholar]
15) Reshetnikov V, Malyutina SK, Nikitin YP. Frequency of the revealings of some infections in patients with an ischemic heart trouble. Clin Med. 2008;2:33–37. [Google Scholar]
16) Alekperov EZ, Nadjarov RN. Modern concepts of the inflammation role in an atherosclerosis. Cardiology. 2010;6:88–91. [PubMed] [Google Scholar]
17) Ivashkin VT. Cellular and molecular biology of the liver inflammation. Russ J GastroenterolHepatolColoproctol. 1998;5:13–17. [Google Scholar]
18) Marevichev MM, Jdanov VS, Galahanov IY. Atherosclerosis depending on alcohol intake in almost healthy men. Cardiovascular Therapy Prof. 2009;8(Suppl 1):226. [Google Scholar]

19) Taheri Sarvtin M, ZandParsa A, Kordbacheh P, Hashemi S, Mahmoudi M, Daie R, *et al*. (2014). Camparison of Oral Candida Flora in Patients with Coronary Atherosclerosis and Healthy People. ZJRMS, 16 (1): 40– 43. [Google Scholar] [Ref list]

20) 20-Campbell LA, Yaraei K, Van Lenten B, Chait A, Blessing E, Kuo CC, Nosaka T, Ricks J, Rosenfeld ME. The acute phase reactant response to respiratory infection with Chlamydia pneumoniae: implication for the patogenesis of atherosclerosis. Microbes Infect. 2010;12:598–606. [PMC free article] [PubMed] [Google Scholar]
21) Epstein SE, Zhou YF, Zhu J. Infection and atherosclerosis: emerging mechanistic paradigms. Circulation. 1999;100:e20–8. [PubMed] [Google Scholar]
22) Bayramova GR. Chronic relapsing vulvovaginal candidiasis: the diagnostic principles and therapy possibilities. Obstetrics Gynecology. 2008;6:64–66. [Google Scholar]