International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 25-07-2023, Accepted: 10-08-2023, Published: 26-08-2023 Volume 5, Issue 3, 2023, Page No. 34-36

Bacterial diversity in coronary atherosclerosis patients: A study in Mosul, Iraq

Haqy Yazan Ismail[1], Saba Abdul Salam Hamid Al-Sultan[2*]
[1] College of Science, University of Mosul, Mosul, Iraq
[2] Department of Microbiology, College of Medicine, Ninevah University, Mosul, Iraq

Abstract
Coronary Atherosclerosis has a complicated etiology that is affected by risk factors such as hereditary and environmental variables. Chronic infection and inflammation have been identified as pathogenic factors for developing Coronary Artery Disease (CA). This study aimed to identify the bacterial species that could contribute to the occurrence of atherosclerosis in patients who underwent cardiac catheterization of the coronary arteries in the period from 11/28/2022 to 4/15/2023 from the Mosul Center for Cardiology. and Cardiac Surgery (MCCCS).
Balloon samples were transferred by Tryptone Soy Broth transport medium directly to the laboratory, inoculated on primary isolation media (nutrient agar, blood agar, and MacConkey agar), and incubated at 37 °C for 24 hours in aerobic condition. This study showed that from 100 balloon samples isolating different species of bacteria, 21 positive samples were obtained, and They represented 15 types, according to the Vitek-2 compact system diagnosis. Isolating mixed types of bacteria from atherosclerotic patients is an essential step in understanding the potential role of bacterial infections in atherosclerosis, and can investigate whether these bacteria promote inflammation, interact with lipids, or trigger immune responses, all of which could play a role in the development and progression of atherosclerosis. In conclusion, it was found that many types of bacteria can be isolated from atherosclerosis, and it is believed that the different isolated species are associated with different risk factors that differ according to the patient, immunity, genetic predisposition, and other environmental conditions surrounding him.

Keywords: Bacterial infection, coronary artery disease, atherosclerosis, balloon, vitek-2 compact

Introduction

Atherosclerotic illnesses, such as myocardial infarction and stroke, killed About 17.9 million people each year globally, making atherosclerosis the leading cause of death. Atherosclerosis is thought to be caused by hereditary and environmental causes. In humans, clinical problems do not appear for several decades. Smoking, high blood pressure**,** diabetes, and hypercholesterolemia are all established risk factors for atherosclerosis. However, it is commonly believed that these risk factors interact with the arterial wall cells to generate chronic vascular inflammation, leading to atherosclerosis. Over the last 30 years, genetically engineered mice have been used to study the molecular pathways underlying the pathogenesis of atherosclerosis in great detail. Lipid-lowering medications like statins are thought to be the most effective at preventing and treating atherosclerosis**.** Despite this progress, uncertainties about the pathophysiology of atherosclerosis persist, and new animal models and innovative treatments are needed to treat patients that statins cannot effectively treat. This review will concentrate on two aspects of atherosclerosis, "pathology" and "pathogenesis," and will address unsolved problems (Björkegren and Lusis, 2022; Kandi *et al*., 2016) [1, 2].

According to WHO epidemiological estimates from 2016, Saudi Arabia (46%) and Kuwait (41%) had the highest prevalence rates of ischemic heart disease (Kalaf *et al*., 2016) [3]. Compared to other Eastern Mediterranean countries, Due to the lack of surveillance studies and evidence-based national recommendations for managing cardiovascular disease in Iraq, epidemiological data on the incidence and prevalence of Coronary Artery Disease (CAD) as a measure of awareness are few. (Fuster *et al*., 2005) [4]**.** In Iraq, 33% of fatalities are attributable to cardiovascular disease, according to 2018 research (Tehrani-Banihashemi *et al*., 2018) [5]. To better treat and prevent this illness, it is essential to have a better knowledge of the prevalence of cardiovascular disease and associated risk factors in this area and greater public awareness of CAD symptoms and risk factors (Albustany, 2021) [6]. Numerous studies have shown the presence of bacterial pathogens in atherosclerotic plaque. Pathogens can lie dormant or grow in cells like macrophages, causing a prolonged inflammatory response. Because they operate from within the cell, intracellular bacteria are the most often implicated species (Rosenfeld and Campbell, 2011) [7].

Forty-four studies were chosen. The following bacteria were found in high incidence in coronary arteries: *Chlamydia pneumonia* (42.8%), *Campylobacter rectus* (46.2%), *Cytomegalovirus* (29.1%), *Helicobacter pylori* (18.9%), and *Herpes simplex* virus type 1 are the most common infections (5.9%), *Porphyromonas gingivalis* (42.6%), *Prevotella intermedia* (47.6%), *Tannerella forsythia* (43.7%), and *Treponema denticola* (32.9%) (Razeghian *et al*., 2022) [8]. On the other hand, Research has suggested that some bacteria found in individuals with cardiovascular disease (CVD) may have similar microbial diversity in both periodontal pockets and atheromatous plaques. This association has led to investigations into the potential link between periodontal disease and atherosclerosis**.** However, it is essential to note that the presence and role of bacteria in atherosclerotic plaques still need to be fully understood (Serra *et al*., 2014; Jain *et al*., 2021) [9, 10]. Some of the bacterial species that have been detected in atheromatous plaques include *Chlamydia pneumoniae*, *Porphyromonas gingivalis*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*,

Streptococcus mutants, Escherichia coli, Prevotella intermedia, Campylobacter rectus, and *Fusobacterium nucleatum* (Dinakaran *et al*., 2012) [11]. This study aimed to identify the bacterial species that could contribute to the occurrence of atherosclerosis in patients who underwent cardiac catheterization of the coronary arteries in the period from 11/28/2022 to 4/15/2023 from the Mosul Center for Cardiology. and Cardiac Surgery (MCCCS).

Materials and Methods

From 11/28/2022 to 4/15/2023 samples were taken from patients who underwent PCI (100 balloons samples during angioplasty). A special questionnaire was developed for each patient in MCCCS that contained (constitutional factors, major risk factors, minor risk factors, symptoms, and required laboratory tests). Angioplasty balloon samples were gathered and placed in sterile transport media (TSB) and transferred to the lab directly for investigating microbial and conducting other tests.

The samples in the transport medium were cultured on medium using the method of quadruple streaking and sterilization between each streaking to obtain a pure culture, and they were incubated at 37°C for 24 h in aerobic condition on each of the three different media, blood agar, MacConkey agar, nutrient agar. Isolates growing on primary isolation cultures (nutrient agar, blood agar, and MacConkey agar medium) were diagnosed, In terms of the shape, texture, odor, and color of pure colonies on the culture media used, in addition to their ability to ferment lactose or not on MacConkey agar, and their ability to hemolysis blood on blood agar medium and the type of hemolysis. Direct smears were made for each sample before inoculated on culture media and from pure colonies after growing on culture media and stained with a differential Gram stain, then they were examined microscopically for cell arrangement and shape and to determine whether they were positive or negative for Gram staining, in addition, the most important biochemical tests such as oxidase, catalase, and urease were performed for all isolates (Lagier *et al*., 2015) [12]. And confirm diagnosing by using the Vitek-2 compact system**.**

Results

The results of the initial collection of 100 samples of balloon angioplasty, and the number of bacterial isolates was 21 were isolated, 15 samples which diagnosed Vitek-2 compact system.

Table 1: List of Bacterial Species Isolated from Balloon samples and biochemical tests

Microorganisms	NO. of isolates from	%*	Catalase	Oxidase	Urease
Stutzerimonas stutzeri	5	5(23.8)	+	+	-
Pseudomonas aeruginosa	2	2(9.5)	+	+	-
Staphylococcus haemolyticus	1	1(4.76)	+	-	-
Staphylococcus epidermis	2	2(9.5)	+	-	+
Staphylococcus hominis	1	1(4.76)	+	-	+
Staphylococcus lentus	1	1(4.76)	+	+	+
Streptococcus thoraltensis	1	1(4.76)	+	+	+
Streptococcus sanguinis	1	1(4.76)	-	-	+
Enterococcus saccharolyticus	1	1(4.76)	-	-	-
Micrococcus luteus	1	1(4.76)	+	-	+
Kocuria rhizophilia	1	1(4.76)	+	-	+
Kocuria kristinae	1	1(4.76)	+	-	+
Leuconostoc mesenteroides	1	1(4.76)	-	+	+
Leuconostoc pseudomesenteroides	1	1(4.76)	-	+	+
Lactococcus garvieae	1	1(4.76)	-	-	+
Total	21				

*From the total number of samples (from the total number of isolates)

Discussion

The isolates were diagnosed based on their cultural and phenotypic characteristics and biochemical tests (Oxidase, Catalase, and Urease). It was confirmed using the Vitek-2 Compact device and found that the types were on the following ratios, *Stutzerimonas stutzer* was 23.8%, 5 isolates, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* were 9.5% two isolates for each one of them, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus lentus*, *Streptococcus thoraltensis*, *Streptococcus sanguinis*, *Enterococcus saccharolyticus*, *Micrococcus luteus*, *Kocuria rhizophila*, *Kocuria kristina*, *Leuconostoc mensenteriodes*, *Leuconostoc pseudomesenteroides* and *Lactococcus garvieae*, one isolate for each of them with 4.76%.

Various types of bacteria have been identified in atheromatous plaques, which are the characteristic accumulations of fatty deposits, cholesterol, inflammatory cells, and connective tissue within the arteries' walls during atherosclerosis development (Fruchart *et al*., 2004) [13].

The diversity of bacterial types in atherosclerosis plaques can be attributed to several factors. While research in this area is still ongoing and not yet fully understood, there are some potential reasons for the diversity of bacterial infections in atherosclerosis plaques; such as; Source of Infection; Bacteria may enter the bloodstream from various sources, leading to different types of infections in atherosclerotic plaques. For example, oral bacteria like *Staphylococcus* spp. and *Streptococcus* spp. can be introduced into the bloodstream through periodontal disease (gum disease), while *Chlamydia pneumoniae* might enter the bloodstream through respiratory infections. Individual Variation; Each person's immune system and overall health status can influence which bacteria are more likely to colonize atherosclerotic plaques. The plaque's microbial community composition may vary from person to person based on their unique immune response and exposure to

different bacterial species. Plaque Location and Stage; The bacterial diversity in atherosclerosis plaques might vary depending on the location and stage of the plaque. Different regions within the arterial wall may be exposed to varying levels of bacteria from different sources, leading to diverse bacterial populations. Chronic Inflammation; Atherosclerosis is characterized by chronic inflammation within the arterial walls. The inflammatory environment might promote the growth of specific bacteria, allowing them to colonize the plaque and contribute to its progression. Types of drugs; The type of medication can affect the type of bacteria that settle in atherosclerotic plaques. Patient age; is an important factor because each age group is infected with different types of bacteria. Interaction with Other Microorganisms; Bacteria in atherosclerotic plaques might interact with other microorganisms, such as fungi and viruses, influencing the overall microbial diversity within the plaque. Lifestyle and Environmental Factors; Factors like diet, smoking, and exposure to environmental toxins might impact the composition of bacterial communities within the body, including those found in atherosclerotic plaques. Genetic Predisposition; Genetic factors could influence the susceptibility to certain bacterial infections, leading to variations in bacterial diversity within plaques among different individuals. More research is needed to understand the complex interactions between bacteria, the immune system, and other factors contributing to the development and progression of atherosclerosis. As scientific understanding progresses, a deeper understanding of the reasons behind the diversity of bacterial types in atherosclerosis plaques may emerge, potentially leading to new insights into prevention and treatment strategies for cardiovascular disease (Yadav *et al*., 2022; Van Der Meer *et al*., 2008) [14, 15].

In conclusion, the risk of atherosclerosis results from a previous injury to another area of the body, and the severity of the disease is related to risk factors related to the patient himself.

References

1. Björkegren JLM, Lusis AJ. Atherosclerosis: Recent developments. In Cell,2022:185(10):1630-1645. https://doi.org/10.1016/j.cell.2022.04.004
2. Kandi V, Palange P, Vaish R, Bhatti AB, Kale V, Kandi MR, *et al*. Emerging Bacterial Infection: Identification and Clinical Significance of Kocuria Species. Cureus, 2016, 8(8). https://doi.org/10.7759/cureus.731
3. Kalaf H, Almesned A, Soomro T, Lasheen W, Ewid M, AlMohaimeed AA. Cardiovascular disease risk profile among young Saudi women of Al-Qassim, Saudi Arabia: A crosssectional study. International Journal of Health Science,2016:10(1):29-37. https://doi.org/10.12816/0031214
4. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: Part I: Evolving concepts. Journal of the American College of Cardiology,2005:46(6):937-954. https://doi.org/10.1016/j.jacc.2005.03.074
5. Tehrani-Banihashemi A, Moradi-Lakeh M, Elbcheraoui C, Charara R, Khalil I, Afshin A, *et al*. Burden of cardiovascular diseases in the Eastern Mediterranean Region, 1990–2015: findings from the Global Burden of Disease 2015 study. International Journal of Public Health,2018:63:137-149. https://doi.org/10.1007/s00038-017-1012-3
6. Albustany O. Prevalence of acute Myocardial infarction in young patients in Kirkuk city. Kirkuk Journal of Medical Sciences,2021:9(1):47-83. https://doi.org/10.32894/kjms.2021.170072
7. Rosenfeld ME, Campbell LA. Pathogens and atherosclerosis: Update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. Thrombosis and Haemostasis,2011:106(5):858-867. https://doi.org/10.1160/TH11-06-0392
8. Razeghian-Jahromi I, Elyaspour Z, Zibaeenezhad MJ, Hassanipour S. Prevalence of Microorganisms in therosclerotic Plaques of Coronary Arteries: A Systematic Review and Meta-Analysis. Evidence-Based Complementary and Alternative Medicine, 2022. https://doi.org/10.1155/2022/8678967
9. Serra E Silva Filho W, Casarin RCV, Nicolela ELPassos, HM, Sallum AW, Gonçalves RB. Microbial diversity similarities in periodontal pockets and atheromatous plaques of cardiovascular disease patients. PLoS ONE, 2014, 9(10). https://doi.org/10.1371/journal.pone.0109761
10. Jain P, Hassan N, Khatoon K, Mirza MA, Naseef PP, Kuruniyan MS, et al. Periodontitis and systemic disorder—an overview of relation and novel treatment modalities. Pharmaceutics,2021:13(8):1-23. https://doi.org/10.3390/pharmaceutics13081175
11. Dinakaran V, John L, Rathinavel A, Gunasekaran P, Rajendhran J. Prevalence of Bacteria in the Circulation of Cardiovascular Disease Patients, Madurai, India. Heart Lung and Circulation,2012:21(5):281-283. ttps://doi.org/10.1016/j.hlc.2012.02.007
12. Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clinical Microbiology Reviews,2015:28(1):208-236. https://doi.org/10.1128/CMR.00110-14
13. Fruchart JC, Nierman MC, Stroes ESG, Kastelein JJP, Duriez P. New risk factors for atherosclerosis and patient risk assessment. Circulation, 2004, 109(23 SUPPL.). https://doi.org/10.1161/01.cir.0000131513.33892.5b
14. Yadav LB, Vajpeyee A, Vajpeyee M, Tiwari S. Assessment of Bacterial Diversity Associated With,2022:9(1):1-6.
15. Van Der Meer JJ, Van Der Wal AC, Teeling P, Idu MM, Van Der Ende A, *et al*. Multiple bacteria contribute to intraplaque T-cell activation in atherosclerosis. European Journal of Clinical Investigation,2008:38(11):857-862. https://doi.org/10.1111/j.1365-2362.2008.02031.x