Latent Tuberculosis Infection among Patients with Coronary Artery Stenosis: A Case–Control Study

Ahmad Farooq Alsayed Hasanain, Khalid M. El-Maghraby, Ali A. H. Zayed, Amany M. A. Nafee, Sherif M. Abdel-Aal, Sally M. Bakkar

Departments of 1Tropical Medicine and Gastroenterology, 2Cardiology, 3Chest Diseases, 4Microbiology and Immunology, 5Diagnostic Radiology, 6Biochemistry, Faculty of Medicine, Assiut University, Asyut, Egypt

Abstract

Background: The activation of the cell-mediated immune responses by Mycobacterium tuberculosis can promote atherogenesis. Aims: The aim of this study is to determine the frequency of latent tuberculosis infection (LTBI) among patients with coronary artery stenosis (CAS) and to explore the association between LTBI and development of CAS. We conducted a case–control study which included 183 patients who underwent percutaneous coronary angiography (121 patients with CAS and 62 patients without as a control group). Methods: For all the study population, clinical evaluation, tuberculin skin test (TST), imaging studies (including chest radiography and echocardiography), laboratory investigations, and electrocardiography were carried out. Only for the patients with positive TST, QuantiFERON-TB Gold test was performed. Predictors of CAS were identified using univariate analyses (Yates’ corrected Chi-square test or Fischer’s exact test) followed by multivariate analysis (binary logistic regression). Results: Among 29.5% of the study population, LTBI was detected, and among patients with CAS, 56.2% of patients had advanced CAS. After multivariate analysis, it was found that metabolic syndrome (MS) (odds ratio [OR] 3.6, 95% confidence interval [CI] 1.5–22.6, \( P = 0.022 \)) and LTBI (OR 2.5, 95% CI 1.2–17.3, \( P = 0.018 \)) were the predictors of CAS among the study population, while only diabetes mellitus (DM) (OR 1.9, 95% CI 1.1–11.7, \( P = 0.031 \)) was the predictor of advanced CAS. Conclusion: LTBI is associated with the development of CAS. In addition, MS is associated with CAS, while its related disorder, DM, is associated with advanced CAS.

Keywords: Coronary stenosis, latent tuberculosis, metabolic syndrome

Introduction

Cardiovascular disease is the number one cause of mortality in developed countries.[1] Most of the cases of coronary artery disease are caused by atherosclerosis.[2] Risk factors for atherosclerosis include obesity, diabetes mellitus (DM), dyslipidemia, systemic hypertension, older age, male gender, and heredity. Regardless of the trigger for atherosclerosis, the underlying process is inflammation in response to injury.[3] Atherogenesis involves accumulation of lipids within the wall of blood vessels (noninflammatory component), followed by mononuclear cells infiltration (inflammatory component), with proliferation of the smooth muscle cells to ultimately form atheromatous plaques.[4]

Several infectious agents may contribute to the formation of atheromatous plaques,[5] through a long-lasting pro-inflammatory effect on the wall of endothelial cells.[6] Approximately one-third of the world population is infected with Mycobacterium tuberculosis (Mtb); the majority of those infected have latent tuberculosis infection (LTBI). Globally, approximately two billion persons have LTBI, which is a state of asymptomatic, persistent immune response to Mtb antigens.[7] The diagnostic tools for LTBI include tuberculin skin test (TST) and interferon-gamma release assay (IGRA). Compared to TST, IGRA has the advantage of better specificity.[8] Dynamic equilibrium exists between the host immune system and the actively dividing Mtb in patients with LTBI, with subsequent induction of a chronic inflammatory state.[9] In Egypt, BCG vaccination is an integral part of the public health system, and it has been shown to reduce the incidence of tuberculosis.[10]

Access this article online

Quick Response Code:  
Website: www.ijmyco.org  
DOI: 10.4103/ijmy.ijmy_34_18

Address for correspondence: Dr. Ahmad Farooq Alsayed Hasanain, Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Assiut University, Asyut, Egypt. E-mail: af.hasanain@outlook.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms. For reprints contact: reprints@medknow.com

How to cite this article: Hasanain AF, El-Maghraby KM, Zayed AA, Nafee AM, Abdel-Aal SM, Bakkar SM. Latent tuberculosis infection among patients with coronary artery stenosis: A case–control study. Int J Mycobacteriol 2018;7:143-7.
part of the compulsory vaccination program; it causes a state of immunological stress, with subsequent stimulation of the proatherogenic mechanisms.\(^{[10]}\)

Our research hypothesis relies on the possibility that activation of the cell-mediated immune response by Mtb can promote atherogenesis. Several studies investigated the relation between Mtb infection and atherosclerosis, but as far as we know, no previous studies have explored the contribution of LTBI to the development of coronary artery stenosis (CAS). The aim of this study is to determine the frequency of LTBI among patients with CAS compared to those without and to explore the association between LTBI and CAS.

**Methods**

**Study-Subject**

**Study design**

A hospital-based, case–control study was conducted.

**Study location**

The study population was recruited from patients attending the “Cardiac Catheterization Unit” of the Department of Cardiology, Cardiology, and Cardiac Surgery Hospital.

**Study duration**

The study sample was recruited from February 2016 to December 2017.

**Inclusion criteria**

This study included consecutive 183 patients with chest pain suggestive of ischemic heart disease with intermediate-high risk, who underwent percutaneous coronary angiography for diagnosis of coronary artery disease (CAD). The study population included 121 patients with CAS and 62 patients without. Advanced CAS was defined as the presence of one or more of the following: proximal, left anterior descending coronary artery disease ≥95%, two-vessel disease (other than left anterior descending coronary artery) ≥95%, and three-vessel disease of any degree.

**Exclusion criteria**

Pregnant patients, patients younger than 18 years, and patients with clinical, radiological, and/or microbiological evidence of TB and leukopenia were excluded from the study. Furthermore, patients with human immunodeficiency virus (HIV) infection, and skin rash (that can interfere with the interpretation of TST results), and those receiving aspirin, nonsteroidal anti-inflammatory drugs, systemic corticosteroids, immunosuppressant medications, and/or antineoplastic chemotherapy were not enrolled.

**Methods**

For all the study population, clinical evaluation, imaging studies, laboratory investigations, and electrocardiography were carried out. Body mass index (BMI) was estimated according to the following equation: BMI = weight in kilograms/height in meters squared. Overweight was defined as a BMI of 25 kg/m\(^2\) to <30 kg/m\(^2\). Obesity was defined as a BMI of ≥30 kg/m\(^2\). Waist circumference was measured at the midpoint between the lower costal margin and the iliac crest. Imaging studies included chest radiography and echocardiography before undergoing percutaneous coronary angiography. Laboratory investigations included estimation of fasting serum glucose level, lipids profile (fasting serum levels of total cholesterol, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], and triglycerides), complete blood count, estimation of serum level of creatinine, and testing for antibody to HIV. After a minimum of 14 h overnight fasting, venous blood samples were collected. The same laboratory with the standard methods was used for all the laboratory investigations. Glucose intolerance was defined as fasting glucose level of >6.1 to <7 mmol/L, while DM was defined as fasting glucose level of ≥7 mmol/L or more. Dyslipidemia was defined as one or more of the following: Total cholesterol serum level more than 200 mg/dL, LDL-C serum level more than 130 mg/dL, HDL-C serum level ≥27 mg/dL, and triglycerides serum level of ≥165 mg/dL or more.

In addition, TST was performed using purified protein derivative of the human strain of Mtb (\textit{Vacsera}, Egypt) for strict, intradermal injection of five tuberculin units (0.1 mL). When a discrete, pale elevation of the skin (a wheal) 6–10 mm in diameter was detected, the injection was considered correct. The results of TST were recorded after 72 h; induration of 10 mm or more was considered as a positive result.\(^{[11]}\) Only for the patients with positive TST, IGRA (QuantiFERON-TB Gold (QFT-G) test) Cellestis Ltd, Carnegie, Australia) was performed. In specialized blood collection tubes, the whole blood was collected and incubated at 37°C for 24 h. Measurement of interferon-gamma by enzyme-linked immunosorbent assay was performed after centrifugation of the tubes and removal of the plasma. Tuberculin response percentage (based on interferon-gamma response to the Mtb antigen tube) of 30% or more was considered as a positive result. The diagnosis of LTBI relied on positive results of both TST and IGRA.

The diagnosis of the metabolic syndrome (MS) was considered if three or more of the following items were present: (1) waist circumference more than 102 cm for males or more than 88 cm for females; (2) blood pressure reading of 135/85 mmHg or more or receiving therapy for systemic hypertension; (3) high fasting serum levels of glucose (≥110 mg/dL) or receiving therapy for either glucose intolerance or DM; (4) high fasting serum levels of triglycerides (≥150 mg/dL) or receiving therapy for hypertriglyceridemia; and (5) low fasting serum levels of HDL-C <40 mg/dL for males or <50 mg/dL for females or receiving therapy for low HDL-C level.\(^{[12]}\)

**Statistical analysis**

Data were analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics, version 22.0, release 22.0.0.0; IBM Corp, Armonk, New York, US). Results were expressed as mean ± standard deviation or frequency (percentage) as appropriate. The predictors of CAS among the study population
with CAD were identified using univariate analyses (Yates’ corrected Chi-square test or Fischer’s exact test). Multivariate analysis (binary logistic regression) was used to assess the independent effect of each predictor. Multivariate analysis included significant factors with \( P < 0.05 \) in the univariate analyses.

The software G*Power version 3.1.9.2 was used for a post hoc power analysis of the performed Chi-square tests. An arbitrary effect size was chosen for the power analysis, which precisely was a Cohen’s \( w \) statistic of 0.3. This value conventionally corresponds to a medium-sized effect. The power achieved was 0.84.

**Ethical considerations**

The study was conducted after approval of the Clinical Research Ethical Committee of Assiut Faculty of Medicine and was carried out according to the code of ethics of the World Medical Association (Declaration of Helsinki). All the participants signed consent certificate after discussing in detail with the investigators the certificate participants and the study aim. Participants were clearly informed that refusing to participate in the study will not affect having full benefit of the available medical service. Data confidentiality was respected.

**Results**

The demographic, clinical, and laboratory characteristics of the study population are shown in Table 1. The mean age was 62.5 ± 9.9 years (for patients with CAS it was 64.7 ± 11.5 years, while it was 59.1 ± 8.3 for those without). Among the study population, 72.7% were males (70.3% of patients with CAS and 77.4% of those without). LTBI was detected among 29.5% of the study population. Among the study population, TST reading was positive in 68.9%; however, QuantiFERON-TB Gold test was positive only among 29.5% (95% confidence interval [CI] 22.8%–40.1%). Among the 126 patients with positive TST, 54 (42.9%) had a positive QuantiFERON-TB Gold test. The most frequent risk factor for CAS among the study population was tobacco smoking (48.6%).

Among the well-recognized risk factors, tobacco smoking, obesity, DM, dyslipidemia, and MS were associated with CAS compared to those without. In addition, among patients with CAS, the frequency of LTBI was significantly higher compared to those without CAS (35.5% (95% CI 27.6%–43.2%) versus 17.7% (95% CI 13.7%–21.4%), \( P = 0.009 \)). In Table 2, after multivariate analysis of the factors associated with CAS, it was found that MS (odds ratio [OR] 3.6, 95% CI 1.5–22.6, \( P = 0.022 \)) and LTBI (OR 2.5, 95% CI 1.2–17.3, \( P = 0.018 \)) were the predictors of CAS among the study population.

Among patients with CAS, 56.2% had advanced CAS. Table 3 shows the demographic, clinical, and laboratory characteristics of the patients with CAS. The mean age of the patients with advanced CAS was 67.2 ± 12.3, while that of those with nonadvanced CAS was 61.6 ± 10.1. Male gender was more frequent among patients with nonadvanced CAS compared to those without (73.6% vs. 67.6). The factors associated with advanced CAS were systemic hypertension, DM, and LTBI, when comparing the patients with advanced CSA with those with nonadvanced CAS. As shown in Table 4, only DM (OR 1.9, 95% CI 1.1–11.7, \( P = 0.031 \)) was significantly, independently associated with advanced CAS. A flowchart of the study population is shown in Figure 1.

**Discussion**

To the best of our knowledge, it was the first time to find a relation between LTBI and CAS. The previous studies reported an association between LTBI and atherosclerosis but not CAS. On comparison of the study patients with CSA to those without, we found a significant, independent association between LTBI and CAS, referring to a potential contribution of LTBI to the development of CAS through the induction of atherosclerosis; this association was independent of all the other well-recognized risk factors of coronary atherosclerosis. Although a significant association was also detected between LTBI and advanced CAS. In response to injury, the resulting inflammatory process can trigger the atherogenic process; this can lead to the release

### Table 1: Demographic, clinical, and laboratory characteristics of the study population (n=183)

| Characteristic       | CAS (n=121) | No CAS (n=62) | \( P \) |
|----------------------|-------------|---------------|-------|
| Age (years)          | 64±11.5     | 59±18.3       | 0.482 |
| Age ≥40 years        | 104 (86)    | 50 (80.7)     | 0.337 |
| Gender (male)        | 85 (70.3)   | 48 (77.4)     | 0.396 |
| Family history of IHD| 56 (46.3)   | 21 (33.9)     | 0.072 |
| Residence (urban)    | 76 (62.8)   | 29 (46.8)     | 0.064 |
| Tobacco smoking      | 67 (55.4)   | 22 (35.5)     | 0.049*|
| Systemic hypertension| 46 (38)     | 19 (31.7)     | 0.103 |
| Overweight           | 41 (33.9)   | 18 (29)       | 0.421 |
| Obesity              | 35 (28.9)   | 11 (17.7)     | 0.028*|
| Abdominal obesity    | 32 (26.5)   | 12 (19.4)     | 0.115 |
| Glucose intolerance  | 24 (19.8)   | 10 (16.1)     | 0.203 |
| DM                   | 48 (39.7)   | 13 (21)       | 0.006*|
| Dyslipidemia         | 53 (43.8)   | 17 (27.4)     | 0.014*|
| MS                   | 22 (18.1)   | 5 (8.1)       | 0.001*|
| LTBI                 | 43 (35.5)   | 11 (17.7)     | 0.009*|

Data are presented as frequency (%) except for age which is presented as mean±SD. *Statistically significant. CAS: Coronary artery stenosis, IHD: Ischemic heart disease, DM: Diabetes mellitus, MS: Metabolic syndrome, LTBI: Latent tuberculosis infection, SD: Standard deviation

### Table 2: Predictors of coronary artery stenosis among the study population (n=183)

| Predictor | OR  | 95% CI     | \( P \) |
|-----------|-----|------------|-------|
| MS        | 3.6 | 1.5–22.6   | 0.022*|
| LTBI      | 2.5 | 1.2–17.3   | 0.018*|

*Statistically significant. OR: Odds ratio, CI: Confidence interval, MS: Metabolic syndrome, LTBI: Latent tuberculosis infection
of several inflammatory mediators including tumor necrosis factor-alpha and interleukins, with the consequent accumulation of lipids and mononuclear cells (macrophages and activated T lymphocytes within the damaged wall of blood vessels).

Several infectious agents (including viral, bacterial, and parasitic pathogens) were linked to atherogenesis, through both direct and indirect mechanisms. The direct mechanism involves mycobacterial direct invasion of the wall of blood vessels leading to an autoimmune reaction, and subsequently thrombosis.

Indirectly, the cell wall of Mtb can induce a pro-inflammatory state, which may contribute to atherogenesis.

An autoimmune response can be induced against the expressed heat-shock proteins on endothelial cells, macrophages, and smooth muscle cells due to the molecular similarity of the bacterial heat-shock protein-60 to the human shock proteins, leading to atherogenesis.

An autoimmune response can be induced against the expressed heat-shock proteins on endothelial cells, macrophages, and smooth muscle cells due to the molecular similarity of the bacterial heat-shock protein-60 to the human shock proteins, leading to atherogenesis.

A proatherogenic immune response against the BCG vaccine was suggested by finding a correlation between the titers of antibody to heat-shock protein-65 and atherosclerosis.

The antibodies against shared epitopes of heat-shock protein-60 between Mtb and the human cell are involved in the development of atherosclerosis through the induction of an autoimmune process. Mycobacterial wall phospholipids (especially phosphatidylinositol) induces a procoagulant state, through their contribution to thrombin generation.

The previous data explains the role of Mtb in triggering and/or accelerating atherogenesis, with consequent development of CAS.

In addition to LTBI, MS was a predictor of CAS among our study population. Endothelial dysfunction induced by MS can be due to an inflammatory state or oxidative stress. In blood vessels, the endothelium represents a dynamic interface between the arterial wall and the circulating cells in bloodstream. Therefore, endothelial dysfunction is considered as the initial step of atherogenesis. Oxidative stress leads to reduced bioactivity of nitric oxide, which controls the vascular tone, inhibits platelet function, prevents leukocytes adhesion, and reduces intimal proliferation.

Regarding advanced CAS, DM was the only factor significantly, independently associated with such a disorder. This refers to the contribution of DM to the progression of coronary atherosclerosis after being initiated by MS and LTBI among our study population. The main pathways involved in atherogenesis among patients with DM are the enhanced inflammatory response and thrombotic state.

Data are presented as frequency (%) except for age which is presented as mean±SD. *Statistically significant. OR: Odds ratio, CI: Confidence interval, DM: Diabetes mellitus
The present study did not include laboratory assessment of serum insulin for confirmation of insulin resistance; we relied on the clinical presentation of insulin resistance in the form of MS. Another limitation was the relatively smaller size of the control group in comparison to the cases with CAS.

**Conclusion**

LTBI is associated with the development of CAS but not with advanced CAS. In addition, MS is associated with CAS, while its related metabolic disorder, DM, is associated with advanced CAS. Whether screening for LTBI and treating those with infection can reduce the development of CAS needs further studies.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Warburton DE, Bredin SS. Health benefits of physical activity: A systematic review of current systematic reviews. Curr Opin Cardiol 2017;32:541-56.
2. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: A comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20:1262-75.
3. Fong IW. Emerging relations between infectious diseases and coronary artery disease and atherosclerosis. CMAJ 2000;163:49-56.
4. George J, Shoenfeld Y, Afek A, Gilburd B, Keren P, Shaish A, et al. Enhanced fatty streak formation in C57BL/6J mice by immunization with heat shock protein-65. Arterioscler Thromb Vasc Biol 1999;19:505-10.
5. Shoenfeld Y, Sherer Y, Harats D. Atherosclerosis as an infectious, inflammatory and autoimmune disease. Trends Immunol 2001;22:293-5.
6. Shah PK. Link between infection and atherosclerosis: Who are the culprits? Viruses, bacteria, both, or neither? Circulation 2001;103:5-6.
7. Salgame P, Geadas C, Collins L, Jones-Lopez E, Ellner JJ. Latent tuberculosis infection – Revisiting and revising concepts. Tuberculosis (Edinb) 2015;95:373-84.
8. Dodd CE, Schlesinger LS. New concepts in understanding latent tuberculosis. Curr Opin Infect Dis 2017;30:316-21.
9. Huaman MA, Henson D, Ticona E, Sterling TR, Garvy BA. Tuberculosis and cardiovascular disease: Linking the epidemics. Trop Dis Travel Med Vaccines 2015;1. pii: 10.
10. Lamb DJ, El-Sankary W, Ferns GA. Molecular mimicry in atherosclerosis: A role for heat shock proteins in immunisation. Atherosclerosis 2003;167:177-85.
11. Howard A, Mercer P, Nataraj HC, Kang BC. Bevel-down superior to bevel-up in intradermal skin testing. Ann Allergy Asthma Immunol 1997;78:594-6.
12. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel III). JAMA 2001;285:2486-97.
13. Zügel U, Kaufmann SH. Activation of CD8 T cells with specificity for mycobacterial heat shock protein 60 in mycobacterium bovis bacillus calmette-guérin-vaccinated mice. Infect Immun 1997;65:3947-50.
14. Stöllberger C, Finsterer J. Role of infectious and immune factors in coronary and cerebrovascular arteriosclerosis. Clin Diag Lab Immunol 2002;9:207-15.
15. Hoffmeister A, Rothenbacher D, Bode G, Persson K, Márz W, Nauck MA, et al. Current infection with helicobacter pylori, but not seropositivity to chlamydia pneumoniae or cytomegalovirus, is associated with an atherogenic, modified lipid profile. Arterioscler Thromb Vasc Biol 2001;21:427-32.
16. Xu Q, Kiechl S, Mayr M, Metzler B, Egger G, Oberhollenzer F, et al. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis: Clinical significance determined in a follow-up study. Circulation 1999;100:1169-74.
17. Zhu J, Quyyumi AA, Rott D, Csako G, Wu H, Halcox J, et al. Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: Evidence for an autoimmune component of atherogenesis. Circulation 2001;103:1071-5.
18. Lamb DJ, Ferns GA. The magnitude of the immune response to heat shock protein-65 following BCG immunisation is associated with the extent of experimental atherosclerosis. Atherosclerosis 2002;165:231-40.
19. Perschinka H, Mayr M, Millonig G, Mayerl C, van der Zee R, Morrison SG, et al. Cross-reactive B-cell epitopes of microbial and human heat shock protein 60/65 in atherosclerosis. Arterioscler Thromb Vasc Biol 2003;23:1060-5.
20. Rota S, McWilliam NA, Baglin TP, Byrne CD. Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: Modulation by oxidation and vitamin E. Blood 1998;91:508-15.
21. Caballero AE. Endothelial dysfunction, inflammation, and insulin resistance: A focus on subjects at risk for type 2 diabetes. Curr Diab Rep 2004;4:237-46.
22. Santilli F, Vazzana N, Liani R, Guagnano MT, Davi G. Platelet activation in obesity and metabolic syndrome. Obes Rev 2012;13:27-42.
23. Nikolopoulou A, Kadoglou NP. Obesity and metabolic syndrome as related to cardiovascular disease. Expert Rev Cardiovasc Ther 2012;10:933-9.
24. Rafikov R, Fonseca FV, Kumar S, Pardo D, Darragh C, Elms S, et al. ENOS activation and NO function: Structural motifs responsible for the posttranslational control of endothelial nitric oxide synthase activity. J Endocrinol 2011;210:271-84.
25. Pechlivanis N, Ajan RA. Thrombosis and vascular inflammation in diabetes: Mechanisms and potential therapeutic targets. Front Cardiovasc Med 2018;5:1.