Glycemic control in type 2 diabetes mellitus prevents coronary arterial wall infection

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Original Article

Abstract

BACKGROUND: Diabetes mellitus (DM) is a very well-known risk factor for development of atherosclerosis, and it has been hypothesized that poor glycemic control and hyperglycemia plays a major role in this process. In the current study, we aimed to evaluate the associates of poor glycemic control in Iranian patients who have already undergone coronary artery bypass grafting (CABG), with especial focus on the inhabitation of infectious agents within the coronary arterial wall.

METHODS: In January 2010, 52 consecutive patients with type 2 DM who undergone CABG at the Department of Cardiovascular Surgery of Baqiyatallah University of Medical Sciences (Tehran, Iran) were included into this cross-sectional study and biopsy specimens from their coronary plaques were taken and analyzed by polymerase chain reaction (PCR) methods for detecting *Helicobacter* species, cytomegalovirus (CMV) and *Chlamydia pneumoniae*, and their potential relation to the glycemic control status in these patients.

RESULTS: Compared to that in diabetic patients with mean fasting blood sugar (FBS) levels FBS < 126, atherosclerotic lesions in type 2 diabetic patients with poor glycemic control (FBS > 126) were significantly more likely to be positive for CMV PCR test (41% vs. 9%, respectively; P = 0.05). In laboratorial test results, mean triglyceride level was significantly higher among patients of poor glycemic control (168 ± 89 vs. 222 ± 125 mg/dl, respectively; P = 0.033). Hypertension was also significantly more prevalent in this population (73% vs. 36%, respectively; P = 0.034).

CONCLUSION: Type 2 diabetic patients with poor glycemic control can be at higher risk for developing CMV infection in their coronary arterial wall, which can promote atherosclerosis formation process in this patient population. According to the findings of this study, we recommend better control of serum glucose levels in type 2 diabetic patients to prevent formation/progression of atherosclerosis.

Keywords: Diabetes Mellitus, Hyperglycemia, Coronary Artery, Cytomegalovirus, Infection

Introduction

Coronary artery disease (CAD) is the one of the leading cause of mortality and morbidity all over the world, which represents a high fatality highlighting the need for evaluating risk factors and associations. Diabetes mellitus (DM) is forerunning risk factor for cardiovascular disease (CVD) development and one of the best known predictive factors for mortality in this patient population especially among women.[1,2]

In patients with type 2 DM previous prospective studies have shown an association between the degree of hyperglycemia and increased risk of cardiovascular complications.[3,4] It has been demonstrated that poor glycemic control in diabetic patients is associated with clinical complications including the myocardial infarction.[5]

Patients with DM have a two-fold to three-fold increased incidence of diseases related to atheroma; nevertheless, factors inducing an excessive risk for diabetes in inducing CVD or its clinical

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complications are not fully revealed; although hyperglycemia as well as diabetic dyslipidemia is likely to be major contributor.[6] For patients with type 1 DM, previous prospective studies have shown an association between glycemia and the progression of cardiovascular complications and deaths.[7] Patients with type 2 DM have high levels of dyslipidemias, including increased levels of triglyceride, decreased levels of high-density lipoprotein (HDL) cholesterol, and smaller absolute elevations of low-density lipoprotein (LDL) cholesterol levels relative to non-diabetic patients.[8]

It has been suggested that atherosclerosis is primarily a chronic inflammatory disease. A potential link between infectious agents and atherosclerosis has been debated for more than a century.[9] After the introduction of Coxsackie B4 virus, as a possible infectious etiology of coronary arteritis,[10] several other infective agents have been reportedly detected in the arterial plaque samples such as herpes simplex virus (HSV) and cytomegalovirus (CMV). The first bacterium that was proposed to play a role in the coronary artery diseases and myocardial infarction is *Chlamydia pneumoniae*[11] and since then, several other studies were conducted to determine potential roles of bacterial infections such as the *C. pneumoniae* and other agents in the development of coronary atheromas among, which periodontal microbes and *Helicobacter pylori* were more frequently studied.[12]

Patients with DM are at an increased risk for CAD and frequently require coronary artery bypass grafting (CABG). However, few studies have been conducted to investigate that whether this risk enhancement is due to an increased risk of inhabitation of infection within the coronary arterial walls or can it be attenuated with a better control of hyperglycemia? In the current study, which we believe that is the first in the current literature, the relationship between poor glycemic control in patients with type 2 DM and different factors, including infective agents' inhabitation in the coronary arterial wall, has been evaluated in our CABG patient population.

Materials and Methods

Study participants

Until January 2010, 52 consecutively selected type 2 DM patients who were admitted to the Department of Cardiovascular Surgery of Baqiyatallah University of Medical Sciences (Tehran, Iran) with different complaints and manifestations of ischemic heart disease, and who underwent CABG surgery were included into this cross-sectional study and biopsy specimens from their coronary plaques were taken and analyzed for existence of atherosclerotic plaques and DNA of *Helicobacter* species (H. spp.), *C. pneumoniae*, and CMV. Inclusion criteria included: (1) cardiovascular patients on the list of CABG, (2) they should have documented history of type 2 diabetes, and (3) they should be under observation for their diabetes. Diagnosis of type 2 DM was performed when fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or 2-h plasma glucose ≥ 11.1 mmol/l (200 mg/dl), according to the current WHO definition criteria, in all patients.

Data acquisition

Data on demographics, smoking habit, lipid profile, and medical history were recorded for all subjects. Acute coronary syndrome was defined as positive documented history for previous episode(s) for unstable angina and/or myocardial infarction. A positive "family history" was defined when a positive history was reported on at least one first and/or second family members before the age of 56.[13]

Definition of the study groups

For stratifying fasting blood sugar (FBS) levels of our patients as high and normal, we used two approaches: in the first and the second approach, cut-off points of 110 (Stratus I) and 126 (Stratus II) mg/dl were used for categorization. In the third approach, patients with FBS levels of lower than 110 and higher than 126 were compared (Stratus III) (data not shown).

Laboratorial evaluations

Fibrinogen concentrations were measured by using the Clauss method with commercial reagents (Mahsa-yaran, Tehran, Iran). C-reactive protein (CRP) was measured by turbidimetric method. Lipid concentration levels were determined using standard procedures at Baqiyatallah Hospital Central Laboratory (Tehran, Iran) for all patients.

Polymerase chain reaction (PCR)

Tissue samples were dissected in the operating room and stored under sterile conditions. Atherosclerotic plaques were confirmed and reported by a pathologist for all the specimens. Artery specimens were placed in microcentrifuge tubes without using binding buffer. Transport vials were sealed in the operating room and opened only in the laminar airflow safety cabinet at the microbiology laboratory. All of the specimens were kept at $-20°$ C until processing. For preparation of genomic DNA and polymerase chain reaction (PCR), DNA was extracted from endoarterectomy specimens by using the QIAamp tissue mini-kit (Qiagen Inc., Valencia, CA, USA). The DNA absorbed in the QIAamp spin column was eluted

with 55 μL of Tris-EDTA and then subjected to the PCR. DNA from each of the three infective agents were extracted using a commercially available kit (Qiagen, Hilden, Germany) and analyzed using conventional methods.

Ethical considerations

This study was approved by the Local Committee of the University Research Review Board (URRB) of Baqiyatallah University of Medical Sciences (Tehran, Iran). All subjects provided written informed consent to participate in the study and were assured that their personal information will remain anonymous and confidential.

Statistical analysis

Data were analyzed using SPSS for Windows (SPSS 17.0, Corp., Chicago, IL, USA). Chi-square test, Student's t-test, and Kruskal-Wallis test were used where appropriate. All statistical analyses were performed at the 0.05 significance level.

Results

Characteristics of the study participants are summarized in table 1. Twenty-eight (53.8%) of the study population were males, and the remaining 24 (46.2%) were females. Mean age at surgery was 61.0 ± 8.9 years old. Mean body mass index (BMI) was 27.7 ± 3.4 kg/m². Mean FBS was 187.0 ± 70.4 mg/dl. According to Stratus I, 49 (94%) of patients had out of range FBS; while based on the second and third strata 41 (79% and 93%, respectively) had FBS over 126 mg/dl.

A history of unstable angina or myocardial infarction was confirmed in 20 (38.5%) patients. The number of patients whose biopsy specimens from atherosclerotic lesions were reported positive by PCR analysis for H. spp., CMV, and *C. pneumoniae* were 13 (25.0%), 16 (30.8%), and 8 (15.4%), respectively.

When FBS cut-off point has been defined at the level of 110 mg/dl, no significant difference has been observed regarding any of the study variables, therefore, the cut-off was raised to 126 mg/dl; and patients with in-range (< 126) and out-range (> 126) FBS were comparable regarding their age (P = 0.761), mean weight (P = 0.743), mean BMI (P = 0.477), mean fibrinogen value (P = 0.639), mean cholesterol total (P = 0.635), mean LDL levels (P = 0.979), and mean HDL levels (P = 0.674). Moreover, patients of the two groups were comparable regarding gender (P = 0.190; χ^2 = 2.00), CRP positive rate (P = 0.705; χ^2 = 0.54), smoking (P = 0.424; χ^2 = 0.58), and acute coronary syndromes (P = 0.393; χ^2 = 1.17). Prevalence of positive PCR tests for H. spp. and *C. pneumoniae*

Table 1. Comparison of the study parameters in diabetic patients with or without proper glycemic control (fasting blood sugar cut-off: 126 mg/dl)

Parameters	Stratus II (cut-off FBS = 126 mg/dl)		
	In range	Out range	P
Mean age ± SD (year)	60.5 ± 9.8	59.9 ± 8.9	0.761
Mean weight ± SD (kg)	75.3 ± 11.6	76.0 ± 10.0	0.743
Mean BMI ± SD (kg/m^2)	27.5 ± 3.3	28.0 ± 3.7.0	0.477
Biochemical examinations			
Triglyceride (mean ± SD)	168.3 ± 89.3	221.9 ± 124.7	0.033
Fasting blood glucose (mean ± SD)	102.6 ± 12.7	199.1 ± 64.9	-
Fibrinogen (mean ± SD)	207.2 ± 48.1	200.8 ± 58.9	0.639
Cholesterol total (mean ± SD)	174.8 ± 41.4	179.3 ± 42.7	0.635
LDL cholesterol (mean ± SD)	98.4 ± 38.2	98.2 ± 37.8	0.979
HDL cholesterol (mean ± SD)	41.8 ± 12.5	42.9 ± 10.2	0.674
CRP (%)	12 (29.3)	2 (18.2)	0.705
Medical history			
Hypertension (%)	4 (36.4)	30 (73.2)	0.034
Smoking (%)	7 (17.1)	3 (27.3)	0.424
Acute coronary syndromes (%)	3 (42.9)	17 (65.4)	0.393
PCR test positive (%)			
CMV	1 (9.1)	17 (41.5)	0.050
Helicobacter spp.	8 (72.7)	31 (75.6)	0.562
C. pneumonia	3 (27.3)	5 (12.5)	0.343
Gender male (%)	8 (72.7)	20 (48.8)	0.190

FBS: Fasting blood sugar; SD: Standard deviation; BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; CRP: C-reactive protein; PCR: Polymerase chain reaction; CMV: Cytomegalovirus; C. pneumonia: Chlamydia pneumonia

were also equal in the two groups (P = 0.562; χ^2: 0.38 and P = 0.343; χ^2: 1.52, respectively).

Atherosclerotic lesions in patients with out-range FBS were significantly more likely to be positive for CMV PCR test (P = 0.05; χ^2: 3.08). In laboratorial test results, mean triglyceride level was significantly higher among the out of range patients (P = 0.033). Hypertension was also significantly more prevalent in the out-range FBS group (P = 0.034; χ^2: 5.19).

Discussion

Several authors have recommended a significant role for inflammatory mechanisms in the pathogenesis of coronary artery disease, although due to its unclear mechanisms and associations, existence of such relationship is under doubt. Epidemiological studies have suggested associations between several infective agents affecting coronary arterial wall and atherosclerosis formation.[14,15] On the other hand, DM is one of the best known risk factor for atherosclerosis development. To the best of our knowledge, there is no study in the current literature evaluating a potential association between DM and infective agents complicating coronary artery walls. In the current study, we evaluated any potential association between hyperglycemia due to a poor DM control and three infective agents, H. spp., CMV, and *C. pneumoniae*, in atherosclerosis lesions in our Iranian patient population undergoing CABG.

Several authors have previously investigated associations of the three mentioned infective agents with arterial atherosclerosis. H. spp. are one of the most frequently investigated infections whose associations with atherosclerosis are very well-demonstrated;[16,17] although contradictory results have also been reported.[18] Moreover, several authors have issued *C. pneumoniae* replication in atherosclerotic arterial wall.[19] On the other hand, some others even have suggested a positive relationship between *C. pneumoniae* infection and metabolic disorders, which can provide an explanation for its potential atherosclerogenic activity.[20] The same association was also found for CMV infection.[21] In the current study, we did not find any association between poor glycemic control in type 2 diabetic patients and *C. pneumoniae* or H. spp.; nevertheless, a positive association was found for CMV inhabitation in coronary artery wall. This association provides two hypotheses: CMV can deteriorate glucose control in diabetic patients, or poor glycemic control in type 2 diabetic patients endangers coronary artery to CMV infection.

Previous studies have shown that CMV infection can affect glucose metabolism. Evidence suggests that CMV infection increases glucose uptake.[22,23] On the other hand, both in naïve and transplant pancreatic cells, it has been demonstrated that CMV infection can increase immunogenicity, which can reduce beta-cell survival.[24] This survival disturbance is to such an extent that some studies have suggested a role for CMV infection in inducing type I diabetes;[25] and post-transplant DM.[26] On the other hand, in type 2 diabetes in which beta-cells are alive and functional, a CMV-induced damage can reduce insulin availability and deteriorate glucose control.

There is data scarcity on the second hypothesis, in which poor glycemic control was introduced as the reason for higher CMV infection risk. However, a study on periodontal infective agents found no association between poor glycemic control in type 2 diabetic patients and CMV infection.[27] On the other hand, in a case study, development of CMV associated colitis was reported in a case of diabetic ketoacidosis.[28] In this article, authors proclaim that immunosuppression induced by hyperglycemia-ketoacidosis can be considered as the precedent to infection. Several studies have suggested that hyperglycemia can make infections.[29-31] As we also mentioned above, the association does not make it clear that what of them, hyperglycemia or infection, was the first factor, and which is the consequence.[32]

This study has some limitations. First of all, the sample size is limited; although as a premier study and due to its very strong methodology, we believe that this limitation would not hurt the credibility of our findings. However, future studies can more strongly corroborate or rule out our results.

Conclusion

To the best of our knowledge, our study is the first study that correlates hyperglycemia with CMV infection in the coronary arterial wall in diabetic patients. This study suggests that diabetic patients should undergo strict glycemic control to prevent atherosclerosis promotion. Further studies with prospective approaches are needed to confirm our findings.

Conflict of Interests

Authors have no conflict of interests.

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