CORRELATION BETWEEN PREDIABETES CORONARY ARTERY CALCIFICATION AND CARDIOVASCULAR RISK FACTORS: A 5-YEAR RETROSPECTIVE CASE STUDY

THUNNAWAT WATTANASETH

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science

Department of Anti-aging and Regenerative Medicine

College of Integrative Medicine, Dhurakij Pundit University

Academic Year 2021

ความสัมพันธ์ระหว่างภาวะก่อนเบาหวาน ภาวะหินปูนเกาะเส้นเลือดหัวใจ และปัจจัยเสี่ยงต่อการเกิดโรคหัวใจ : กรณีศึกษาย้อนหลังห้าปี

ธรรณวัฐ วัฒนาเศรษฐ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาหลักสูตรวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาการชะลอวัยและฟื้นฟูสุขภาพ วิทยาลัยการแพทย์บูรณาการ
มหาวิทยาลัยธุรกิจบัณฑิต
ปีการศึกษา 2564

ใบรับรองวิทยานิพนธ์

วิทยาลัยการแพทย์บูรณาการ มหาวิทยาลัยธุรกิจบัณฑิตย์

ปริญญาวิทยาศาสตรมหาบัณฑิต

หัวข้อวิทยานิพนธ์ ความสัมพันธ์ระหว่างภาวะก่อนเบาหวาน ภาวะหินปูนเกาะเส้นเลือดหัวใจและปัจจัยเสี่ยงต่อการเกิดโรคหัวใจ :กรณีศึกษาย้อนหลังห้าปี

เสนอโดย ธรรณวัฐ วัฒนาศรษฐ์

สาขาวิชา วิทยาการชะลอวัยและฟื้นฟูสุขภาพ

กลุ่มวิชา เวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ

อาจารย์ที่ปรึกษาวิทยานิพนธ์ ผู้ช่วยศาสตราจารย์ นายแพทย์มาศ ไม้ประเสริฐ

ได้พิจารณาเห็นชอบโดยคณะกรรมการสอบวิทยานิพนธ์นี้แล้ว

ลงชื่อ ..... ประธานกรรมการ

(ผู้ช่วยศาสตราจารย์พันโทนายแพทย์พิษ สุวรรณทัตาทร)

ลงชื่อ ..... กรรมการและอาจารย์ที่ปรึกษาวิทยานิพนธ์

(ผู้ช่วยศาสตราจารย์ นายแพทย์มาศ ไม้ประเสริฐ)

ลงชื่อ ..... กรรมการและอาจารย์ที่ปรึกษาวิทยานิพนธ์(ร่วม)

(ผู้ช่วยศาสตราจารย์ ดร.นายแพทย์พัฒนา เด็งอำนวย)

ลงชื่อ ..... กรรมการและอาจารย์ที่ปรึกษาวิทยานิพนธ์(ร่วม)

(ดร.นายแพทย์ ภาวิต หน่อไชย)

ลงชื่อ ..... กรรมการ

(ผู้ช่วยศาสตราจารย์ นายแพทย์พันธ์ศักดิ์ ศุกระฉกษ์)

วิทยาลัยการแพทย์บูรณาการ รับรองแล้ว

ลงชื่อ ..... คณบดีวิทยาลัยการแพทย์บูรณาการ

(ผู้ช่วยศาสตราจารย์ ดร.นายแพทย์พัฒนา เด็งอำนวย)

วันที่ 30 เดือน มิถุนายน พ.ศ. 2565

Thesis Title:	CORRELATION BETWEEN PREDIABETES, CORONARY ARTERY CALCIFICATION AND CARDIOVASCULAR RISK FACTORS: A 5-YEAR RETROSPECTIVE CASE STUDY
Researcher:	Thunnawat Wattanaseth
Faculty/Department:	College of Integrative Medicine/Anti-Aging and Regenerative Medicine
Advisors / Co-advisors	Asst. Prof. Dr. Mart Maiprasert, M.D., Asst. Prof. Dr. Patana Teng-Umnuay, M.D., Ph.D., Asst. Prof. Dr. Pansak Sugkraroek, M.D., Dr. Phawit Norchai, M.D., Ph.D.

Abstract

Objective: an objective the study was to evaluate the correlation between prediabetic state (HbA1c 5.7 to 6.4%), cardiovascular risks (determined by Framingham Risk Score: FRS) and coronary artery calcium (CAC) score, by retrospective analysis of 5 years data documents on PACS, Jan 2015 to Dec 2020.

Methods: There were 1,639 eligible cases, reviewed on PACS, divided two groups; (1) prediabetes group, 756 cases and (2) non-diabetes group, 883 cases. All eligible cases were reviewed results of vital signs, BMI, CACS, blood test, HbA1c, Fasting blood sugar (FBS), lipid profiles, and serum uric acid. Linear regression and Adjusted Odd ratio were used as statistical analysis for correlation between variables.

Results: (1) Prediabetes cases ($5.7\% \leq$ HbA1c $\leq 6.4\%$) significantly associated with coronary calcification about 2.38 times to non-diabetic cases (Adjusted Odds Ratio = 2.38886 [95% CI (1.98212 – 14.983800)]., (2) Intermediate cardiovascular risk (FRS) associated with positive coronary artery calcification about 2.36 times to low cardiovascular risk [Multivariate adjusted OR = 2.36 (95% CI (1.06 – 5.46)]., and (3) High cardiovascular risk (FRS) associated with positive

coronary artery calcification about 8.64 times to low cardiovascular risk [Multivariate adjusted OR = 8.64 (95% CI (2.65 – 18.58)].

Conclusion: Prediabetes significantly associated with positive coronary artery calcification. In the case of prediabetes with low to intermediate risk of FRS, the CACS is suitable modality of choice for detection coronary calcification and further planning for prevention and/or treatment of coronary artery disease

Keywords: Prediabetes, Coronary Artery Calcium Score, Framingham Risk Score

Table of Content

Table of Content

Table of Figures

Table of Figures

Table of Table

Table of Table

Chapter I

Introduction

1. Background of problem and significant of the study

Prediabetes is a metabolic stage between normal glucose homeostasis and diabetes It can develop diabetes and cardiovascular disease in the future (American Diabetes Association, 2017). The continuing increase in the prevalence of diabetes mellitus and prediabetes in the general population is predicted to result in a higher incidence of cardiovascular disease. Although the mechanisms of diabetes associated progression of atherosclerosis are not fully understood, at clinical and pathological levels, there is an appreciation of increased disease burden and higher levels of coronary calcification and vascular calcification in patients. The plaques within the coronary arteries of patients with diabetes mellitus generally exhibit larger necrotic cores and significantly greater inflammation consisting mainly of macrophages and T lymphocytes relative to patients without diabetes mellitus. Moreover, there is a higher incidence of healed plaque ruptures and positive remodeling in type 1 diabetes mellitus and type 2 diabetes mellitus, suggesting a more active atherogenic process. Lesion calcification in the coronary artery is high risk for progression to coronary artery stenosis and myocardial ischemia.

Coronary artery calcification in diabetics is associated with larger necrotic core size and greater inflammatory infiltrates of macrophages and T lymphocytes, resulting in more diffuse atherosclerosis. The development of coronary calcification are multiple mechanisms including hyperglycemia-induced increases in oxidative stress, endothelial dysfunction, renal function–induced alterations in mineral metabolism, increased inflammatory cytokine production, and release of osteoprogenitor cells from the marrow into the circulation.

In 2011, the American Diabetes Association (ADA) proposed that glycated hemoglobin A1c (HbA1c) could be used as a diagnostic test for diabetes and prediabetes as an alternative to impaired fasting glucose (TFG) or impaired glucose tolerance (IGT). By HbA1c value of 5.7 –

6.4% was recommended for prediabetes. While, HbA1c value greater than 6.5% was recommended for diabetes. Diabetes relates with microvascular disease, microangiopathy, cerebrovascular disease, coronary artery disease, renal angiopathy, and impairment of renal function. According to report of global heath estimate causes of deaths in 2016 by World Health Organization (WHO), the ischemic heart disease and stroke are the world's biggest killers, accounting for a combined 15.2 million deaths.

In 2018, American Heart Association (AHA) guideline suggests that coronary artery calcium (CAC) testing may be considered in adults 40 -75 years of age without diabetes mellitus and with LDL-C level > 70 to 189 mg/dl at a 10-years atherosclerotic cardiovascular disease (ASCVD) risk of 7.5% to 20% (Ron, 2020). Coronary artery calcium (CAC) score is a strong predictor of coronary heart disease (Agatston et al, 1990; Arad et al, 2005). It is considered as the best modality of choice (Non-Invasive Investigation for Coronary Atherosclerotic Marker) in subclinical coronary artery disease, especially in asymptomatic patients with intermediate cardiovascular risk (Ron, 2020 ; De Malced, 2017)

In present, subclinical prediabetes are increasing in general population which has life style dealing with high glycemic food, high sugar intake, fast foods, oxidative stress, inadequate exercise, and no available time for health checkup. While, conventional medicine focuses only treatment of patients whom diagnoses diabetes, cardiovascular event, or full filled criteria of diseases.

In the role of anti-aging and regenerative medicine, an early detection of subclinical disorders, healthy promotion, prevention of disease, prevention of disease progression, prevention of complication, and enhanced life style modification are key successful paradigms for prediabetes and other subclinical disorders.

On annual Checkup program, the high alert to response subclinical laboratory changes, significant clinical history, and detection of minor abnormal findings on physical examination are important roles.

Finally, aim of the study was to evaluate statistically significant on early detection coronary calcification by CT scan in subclinical prediabetes with low to intermediate Framingham Risk Score (FRS) for cardiovascular disease. The expected result of the study would give significant

information that has clinical impact and effect to awareness and management prediabetic cases in clinical practices.

2. Research Objective

An objective the study was to evaluate the correlation between prediabetic status (HbA1c 5.7 to 6.4%), cardiovascular risks (determined by Framingham Risk Score: FRS) and coronary artery calcium (CAC), by retrospective analysis of 5 years data documents, Jan 2015 to Dec 2020.

3. Statement of the Research Questions

3.1 Is there a statistically significant relationship between CAC and HbA1c in prediabetic cases?

3.2 Is there a statistically significant relationship between CAC and cardiovascular risk factors (determined by FRS) in prediabetic cases?

4. Scope of Research

The study is retrospective case control study which used quantitative method, consist of scopes as follow;

4.1 Scope of Content

The study was focused on coronary artery calcium (CAC) and glycated hemoglobin (HbA1c), and Framingham Risk Score (FRS) in prediabetic and nondiabetic participants without known cardiovascular disease, among core knowledge of related theory, literature reviews and related researches, as follows;

(1) Pathology of coronary calcification and vascular calcification in diabetes mellitus

(2) Coronary lesion morphology in patients with and without diabetes mellitus

(3) Computed tomography of coronary artery calcium Score (CACS)

(4) Patterns and genetics of vascular calcification

(5) Major mechanisms of vascular calcification

(6) Hyperglycemic mechanisms related vascular calcification

(7) Dysregulation of phosphate homeostasis and vascular calcification: The Bone -Kidney-Vascular Axis

(8) Circulating cell theory of vascular calcification

(9) Clinical studies of CVD and bone matrix regulator protein

(10) Cardiovascular health and cardiovascular disease

(11) Risk factors related with cardiovascular disease

(12) Epidemiology of cardiovascular disease and cardiovascular risk factors

(13) Framingham heart study (FHS) and Framingham risk score (FRS)

(14) Recent studies of prediabetic stage and coronary artery calcification

(15) Summarized table of reviewed literatures and variables.

4.2 Scope of Variables

The study had quantitative variables, consist of independent variables (Age, Sex, BMI, HbA1c, systolic blood pressure, total cholesterol, HDL, LDL, triglyceride, and smoking), and a dependent variable (coronary artery calcium score: CACS), and a mediator variable (uric acid)

4.3 Scope of Area

Kasemrad International Hospital, Nonthaburi, Thailand.

4.4 Scope of Time

September – December, 2020

5. Expected outcomes

1. CAC was significant higher score in prediabetic group than non-diabetic group.
2. HbA1c and cardiovascular risk (FRS) can predict CAC in prediabetic stage.

6. Expected benefits

1. Coronary artery calcium score (CACS) can be used as atherosclerosis marker in prediabetic case, HbA1c 5.7 to 6.4%, who had intermediate to high cardiovascular risks (FRS)
2. Recommendation of life style modification in prediabetes with positive CACS, under concepts of anti-aging and regenerative medicine, should be performed in clinical practice for prevent further progression of ischemic heart disease

7. Definition of Terms Used in the Research

Prediabetes is a metabolic stage between normal glucose homeostasis and diabetes. American Diabetes Association (ADA) proposed that glycated hemoglobin A1c (HbA1c) value of 5.7 – 6.4% was diagnosed for prediabetes or a fasting blood sugar level from 100 to 125 mg/dL (5.6 to 7.0 mmol/L) is considered prediabetes (table 1.1 and 1.2). In this study, the inclusion criteria for prediabetic case are HbA1c value of 5.7 – 6.4% and fasting blood sugar less than 126 mg/dl. (ADA guideline, 2017)

Table 1.1 HbA1c ranges for diagnosed diabetic status.

Diabetic status	HbA1c (%)
Normal	Less than 5.7%
Prediabetes	5.7 to 6.4%
Diabetes	6.5% to higher

Source: ADA guideline (2011)

Table 1.2 Fasting Plasma Glucose (FPG) ranges for diagnosed diabetic status.

Diabetic status	FPG (mg/dl)
Normal	Less than 100 mg/dl
Prediabetes	100 to 125 mg/dl
Diabetes	126 mg/dl to higher

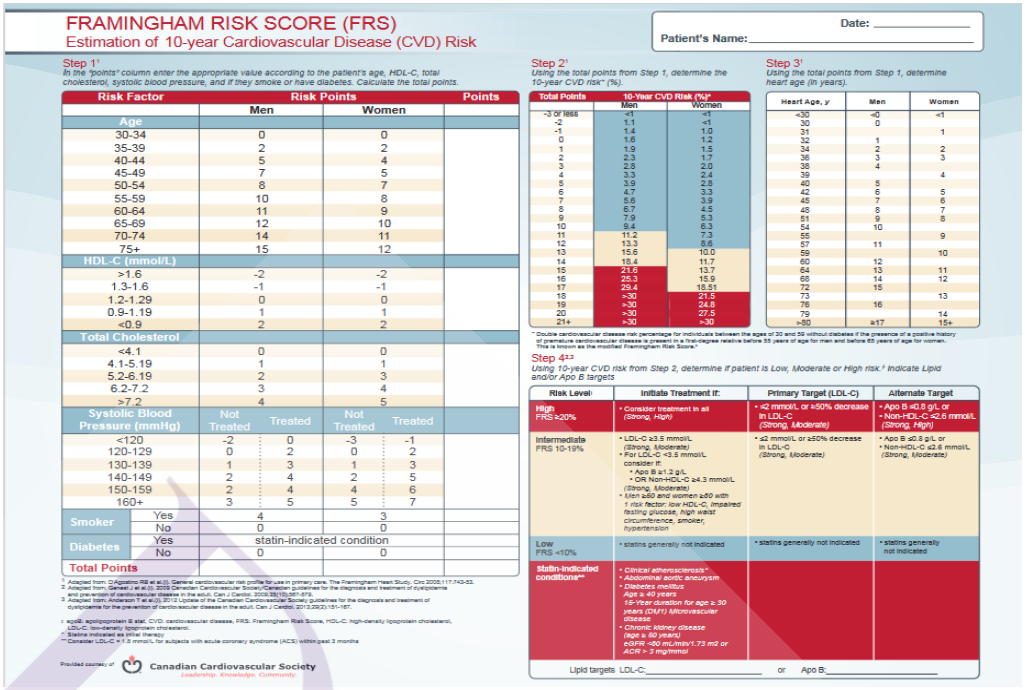
Source: ADA guideline (2011)

Diabetes is metabolic disorders characterized by a high serum blood sugar due to either the pancreas not producing enough insulin (beta cell destruction), or the cells of the body not responding properly to the insulin produced, usually called DM type I and type II, respectively. American Diabetes Association (ADA) proposed that glycated hemoglobin A1c (HbA1c) greater than 6.5% was recommended for diabetes or a fasting blood sugar level greater than 126 mg/dL is considered diabetes. (ADA guideline, 2017)

Nondiabetes is normal glucose homeostasis. American Diabetes Association (ADA) proposed that glycated hemoglobin A1c (HbA1c) value less than 100mg/dl or fasting blood sugar less than 100 mg/dl (ADA guideline, 2017).

Framingham Risk Score (FRS) is a gender-specific algorithm used to estimate the 10-year cardiovascular risk of an individual. The Framingham Risk Score was first developed based on data obtained from the Framingham heart study, to estimate the 10-year risk of developing coronary heart disease. Framingham Risk Score (FBS) used age, gender, total cholesterol, HDL, smoking, diabetic stage, and systolic blood pressure to estimate cardiovascular risk as 10-year risk in percent, as shown on figure 1.1. (ACC/AHA guideline, 2018)

Figure 1.1 Framingham Risk Score (FRS) Chart



FRAMINGHAM RISK SCORE (FRS)

Estimation of 10-year Cardiovascular Disease (CVD) Risk

Date: _____

Patient's Name: _____

Step 1[1]

In the "points" column enter the appropriate value according to the patient's age, HDL-C, total cholesterol, systolic blood pressure, and if they smoke or have diabetes. Calculate the total points.

Risk Factor	Risk Points		Points
	Men	Women	
Age			
30-34	0	0	
35-39	2	2	
40-44	5	4	
45-49	7	5	
50-54	8	7	
55-59	10	8	
60-64	11	9	
65-69	12	10	
70-74	14	11	
75+	15	12	
HDL-C (mmol/L)			
>1.6	-2	-2	
1.3-1.6	-1	-1	
1.2-1.29	0	0	
0.9-1.19	1	1	
<0.9	2	2	
Total Cholesterol			
<4.1	0	0	
4.1-5.19	1	1	
5.2-6.19	2	3	
6.2-7.2	3	4	
≥7.2	4	5	
Systolic Blood Pressure (mmHg)	Not Treated / Treated	Not Treated / Treated	
<120	-2 / 0	-3 / -1	
120-129	0 / 2	0 / 2	
130-139	1 / 3	1 / 3	
140-149	2 / 4	2 / 5	
150-159	2 / 4	4 / 6	
160+	3 / 5	5 / 7	
Smoker Yes	4	3	
Smoker No	0	0	
Diabetes Yes	statin-indicated condition		
Diabetes No	0	0	
Total Points			

Step 2[1]

Using the total points from Step 1, determine the 10-year CVD risk (%).*

Total Points	10-Year CVD Risk (%)* Men	Women
-3 or less	<1	<1
-2	1.1	<1
-1	1.4	1.0
0	1.6	1.2
1	1.9	1.5
2	2.3	1.7
3	2.8	2.0
4	3.3	2.4
5	3.9	2.8
6	4.7	3.3
7	5.6	3.9
8	6.7	4.5
9	7.9	5.3
10	9.4	6.3
11	11.2	7.3
12	13.3	8.6
13	15.6	10.0
14	18.4	11.7
15	21.6	13.7
16	25.3	15.9
17	29.4	18.51
18	>30	21.5
19	>30	24.8
20	>30	27.5
21+	>30	>30

* Double cardiovascular disease risk percentage for individuals between the ages of 30 and 59 without diabetes if the presence of a positive history of premature cardiovascular disease is present in a first-degree relative before 55 years of age for men and before 65 years of age for women. This is known as the modified Framingham Risk Score.[2]

Step 3[1]

Using the total points from Step 1, determine heart age (in years).

Heart Age, y	Men	Women
<30	<0	<1
30	0	
31		1
32	1	
34	2	2
36	3	3
38	4	
39		4
40	5	
42	6	5
45	7	6
48	8	7
51	9	8
54	10	
55		9
57	11	
59		10
60	12	
64	13	11
68	14	12
72	15	
73		13
76	16	
79		14
>80	≥17	15+

Step 4[2]

Using 10-year CVD risk from Step 2, determine if patient is Low, Moderate or High risk.[3] Indicate Lipid and/or Apo B targets

Risk Level	Initiate Treatment If:	Primary Target (LDL-C)	Alternate Target
High FRS ≥20%	• Consider treatment in all *(Strong, High)*	• ≤2 mmol/L or ≥50% decrease in LDL-C *(Strong, Moderate)*	• Apo B ≤0.8 g/L or • Non-HDL-C ≤2.6 mmol/L *(Strong, High)*
Intermediate FRS 10-19%	• LDL-C ≥3.5 mmol/L *(Strong, Moderate)* • For LDL-C <3.5 mmol/L consider if: • Apo B ≥1.2 g/L • OR Non-HDL-C ≥4.3 mmol/L *(Strong, Moderate)* • Men ≥50 and women ≥60 with 1 risk factor: low HDL-C, impaired fasting glucose, high waist circumference, smoker, hypertension	• ≤2 mmol/L or ≥50% decrease in LDL-C *(Strong, Moderate)*	• Apo B ≤0.8 g/L or • Non-HDL-C ≤2.6 mmol/L *(Strong, Moderate)*
Low FRS <10%	• statins generally not indicated	• statins generally not indicated	• statins generally not indicated
Statin-indicated conditions**	• Clinical atherosclerosis* • Abdominal aortic aneurysm • Diabetes mellitus Age ≥ 40 years 10-Year duration for age ≥ 30 years (DM1) Microvascular disease • Chronic kidney disease (Age ≥ 40 years) eGFR <60 mL/min/1.73 m2 or ACR > 3 mg/mmol		

Lipid targets LDL-C:_____ or Apo B:_____

1 Adapted from: D'Agostino RB et al.(1). General cardiovascular risk profile for use in primary care. The Framingham Heart Study. Circ 2008;117:743-53.

2 Adapted from: Genest J et al.(2). 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult. Can J Cardiol. 2009;25(10):567-579.

3 Adapted from: Anderson T et al.(3). 2012 Update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. Can J Cardiol. 2013;29(2):151-167.

† apoB, apolipoprotein B etal, CVD, cardiovascular disease, FRS, Framingham Risk Score, HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol.

* Statins indicated as initial therapy.

** Consider LDL-C <1.8 mmol/L for subjects with acute coronary syndrome (ACS) within past 3 months

Provided courtesy of Canadian Cardiovascular Society

Leadership. Knowledge. Community.

Source: ACC/AHA guideline (2018)

Table 1.3 Framingham Risk Score (FRS)

Category 1: FRS ≤ 10%	Low risk for cardiovascular disease (CVD)
Category 2: 10% ≤ FRS ≤ 19%	Intermediate risk for CVD
Category 3: FRS ≥ 20%	High risk for CVD

Coronary Artery Calcium Score (CACS) is quantitative calculated value of coronary calcification under computed tomography (CT) scan, by using Agatston score (table 1.4), consist of five classified levels, CAC = 0, CAC is 1- 10, CAC is 11-100, CAC is 101-400, and CAC > 400. (ACC/AHA guideline, 2018)

Table 1.4 Interpretation for coronary artery calcium score (CACS)

Calcium Score	Interpretation	Risk for myocardial infarction / Stroke at 10 years
0	Very low risk	< 1%
1-10	Low risk	< 10%
11-100	Moderate risk	10 – 20%
101-400	High risk	15 – 20%
>400	Very high Risk	>20%

Source: ACC/AHA guideline (2018)

Glycated Hemoglobin A1c (HbA1c) is a form of hemoglobin (Hb) that is chemically linked to a sugar. Most monosaccharides including glucose, galactose, and fructose, spontaneously bond with hemoglobin, when present in the bloodstream of humans. The formation of the sugar-Hb linkage indicates the presence of excessive sugar in the bloodstream, often indicative of diabetes. A1C is of particular interest because it is easy to detect. The process by which sugars attach to Hb is called glycation. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin. A1c is measured primarily to determine the three-month average blood sugar level and can be used as a diagnostic test for diabetes mellitus and as an assessment test for glycemic control in people with diabetes. (ADA guideline, 2011)

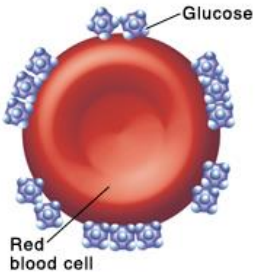


Figure 1.2 Hemoglobin A1C (HbA1C) which glucose sticks to protein (hemoglobin) in red blood cell.

Source: ADA guideline (2017)

CHAPTER 2

Review Literature and Research Works Concerned

Introduction

This chapter presents related theory, literature, and research works concerned about cell biology, physiology, pathology, and clinical aspect of coronary calcification, vascular calcification, atherosclerosis in diabetes, prediabetes, and non-diabetes. The available literature has been presented to the gaps in knowledge to supplement the primary objective of the study, as follows;

2.1 Pathology of coronary calcification and vascular calcification in diabetes mellitus

2.2 Coronary lesion morphology in patients with and without diabetes mellitus

2.3 Computed tomography of coronary artery calcium Score (CACS)

2.4 Patterns and genetics of vascular calcification

2.5 Major mechanisms of vascular calcification

2.6 Hyperglycemic mechanisms related vascular calcification

2.7 Dysregulation of phosphate homeostasis and vascular calcification: The Bone-Kidney

– Vascular Axis

2.8 Circulating cell theory of vascular calcification

2.9 Clinical studies of CVD and bone matrix regulator protein

2.10 Cardiovascular health and cardiovascular disease

2.11 Risk factors related with cardiovascular disease

2.12 Epidemiology of cardiovascular disease and cardiovascular risk factors

2.13 Framingham heart study (FHS) and Framingham risk score (FRS)

2.1 Pathology of coronary calcification and vascular calcification in diabetes mellitus

Vascular calcification can be morphologically classified into 2 distinct forms depending on the location within the intima or the media. Medial calcification is the most common form of vascular calcification in type 2 diabetes mellitus (T2DM), but it mostly affects the media of peripheral arteries, smooth muscle cells and the elastic membrane, resulting in the loss of elasticity.

In this review, the study focuses primarily on the pathological findings in type 1 diabetes mellitus (T1DM) or T2DM with regards to atherosclerotic disease and vascular calcification, mostly intimal (atherosclerotic), which is the dominant type of calcification seen in coronary and carotid diseases. There are important differences in plaque morphology and patterns of calcification in diabetes and without diabetes. There are mechanisms which the metabolic and hormonal abnormalities diabetes mellitus and vascular calcification.

Kazuyuki Yahagi and colleague (Kazuyuki et al, 2017) has been shown pathological findings in sudden coronary death in patients with and without diabetes mellitus by autopsy studies of 438 sudden death victims with diabetes mellitus (n=101) and without diabetes mellitus (n=337) that failed to show differences in the incidence of acute coronary thrombosis (53%) and stable coronary disease (47%). However, the most frequent cause of acute thrombosis was plaque rupture (65%), followed by erosion (30%), and eruptive calcified nodule (5%). Coronary lesions from subjects with T1DM (n=25) and T2DM (n=76) compared with 337 nondiabetic controls showed a lower incidence of acute thrombi (P=0.001 and P=0.02, respectively; Table 2.1, which consistent with previous study (Burke et al, 2004). Likewise, ruptures and erosions were numerically lower in subjects with diabetes mellitus (both T1DM and T2DM) than in nondiabetic subjects, although in some cases, this did not reach statistical significance likely because of the small number of cases (Table 2.1). However, there was a significantly greater plaque burden for those with T1D (P=0.001) and T2D (P=0.02) than for nondiabetic subjects (Table 2.2).

Table 2.1 Distribution of culprit plaques in sudden coronary death relative to diabetic status (438 Cases)

	Type 1 DM (25 Cases)	Type 2 DM (76 Cases)	Non-DM (337 Cases)	*P* Value	
				Type 1 DM vs Non-DM	Type 2 DM vs Non-DM
Acute thrombi	4 (17)	35 (45)	196 (58)	0.001	0.02
Rupture	3 (13)	24 (31)	127 (38)	0.09	0.36
Erosion	2 (8)	7 (9)	61 (18)	0.28	0.06
Calcified nodule	1 (4)	2 (3)	8 (2)	0.48	1.00
Stable CAD	19 (83)	43 (55)	141 (42)	0.001	0.02
CTO	8 (35)	14 (18)	51 (15)	0.04	0.49
No thrombi	11 (48)	29 (37)	90 (27)	0.07	0.05

Source: Yahagi et al (2015)

Table 2.2 Sudden death registry data of coronary plaque characteristics relative to diabetic status

	Type 1 DM (n=16)	Type 2 DM (n=50)	Non-DM (n=66)	*P* Value	
				Type 1 DM vs Non-DM	Type 2 DM vs Non-DM
Necrotic core area, %*	12.0±5.7	11.6±8.4	9.4±9.3	0.05	0.004
Macrophage plaque area, mm^2	0.15±0.02	0.13±0.03	0.10±0.02†	0.03	0.03
Calcified matrix area, %*	7.8±9.1	12.1±11.2	11.4±13.5	0.9	0.05
Fibroatheroma (n)	7.1±5.0	8.8±4.3	6.9±4.7	0.9	0.02
Thin-cap fibroatheroma (n)	1.0±1.3	0.8±0.8	0.7±0.8	0.5	0.8
Healed plaque rupture (n)	2.6±2.1	2.6±1.8	1.9±1.8	0.2	0.04
Total plaque burden, %	275±129	358±114	232±128	0.04	0.0001
Distal plaque burden, %	310±114	630±263	331±199	0.8	0.0001

Values are expressed as mean ± SD or % normalized to plaque area. DM indicates diabetes mellitus.

*P values calculated using log-normalized data.

†P=0.006 vs type 1 and 2 DM combined.

Source: Burke et al (2004)

Overall, in men, the number of plaque ruptures versus erosions was significantly higher ($P<0.05$), whereas erosions were more frequent in females versus ruptures ($P<0.05$), consistent with previous study (Burk et at, 2004). Similar sex-based trends for diabetic subjects were also noted for ruptures and erosions as well although differences did not achieve statistical significance likely because of the limited number of cases.

Devies (1997) studied incidence of coronary plaque rupture of autopsy cases that shown coronary thrombi in 84% of men and 59% of women without diabetes mellitus when compared with only 34% of diabetic patients of either sex. Although high estrogen levels have been suggested as the underlying reason for the differences in plaque morphology between men and women, the underlying reason why diabetics show lower incidence of acute coronary thrombi whereas having greater plaque burden remains unknown but raises the questions of whether the mechanisms of plaque progression may be fundamentally different in diabetic versus nondiabetic patients and even between T1DM and T2DM patients. Although cardiovascular risk in T1DM is driven by hyperglycemia, the cause of atherosclerosis in T2DM is multifactorial, featuring several factors largely absent in T1DM such as obesity, dyslipidemia, and hypertension. The lower incidence of luminal thrombi at autopsy in diabetics is somewhat surprising given the increased platelet reactivity seen in patients with versus without diabetes mellitus. Factors such as increased blood osmolarity (caused by hyperglycemia), hyperglycemia-induced activation of protein kinase C (PKC) β, and greater expression of glycoprotein (Gp IIb/IIIa) and GpIb have been implicated in the effect of diabetes mellitus on platelet function (Schneider, 2009).

2.2 Coronary Lesion Morphology in Patients with and without Diabetes Mellitus

Burke and colleague (Burke at al, 2004) analyzed the impact of diabetes mellitus on lesion morphology in the same autopsy cohort as mentioned above found that necrotic core size and inflammation characterized by macrophages and T cells were significantly greater in subjects with T1DM and T2DM than in nondiabetic controls (Figure 2.1). However, calcified matrix area was greater only in patients with T2DM (12.1±11.2%) but not in patients with T1DM (7.8±9.1%) than in nondiabetics (11.4±13.5%, P versus T1DM=0.9; P versus T2DM=0.05). Moreover, the overall percentage of inflammation relative to total lesion burden was also significantly greater in diabetics

with regards to macrophages (T2DM; P=0.04), T lymphocytes (T1DM; P=0.03), and human leukocyte antigen-DR (T1DM and T2DM; P=0.03 and P<0.0001), respectively (Figure 1). In addition, a multivariate analysis determined that HbA1c was an independent predictor of necrotic core size (T=2.8; P=0.005) and macrophage area (T=2.9; P=0.004) after adjusting for high-density lipoprotein cholesterol, total cholesterol/high-density lipoprotein cholesterol ratio, age, smoking, and sex. Thus, these data suggest differential mechanisms of plaque growth in diabetic versus nondiabetic patients and a link between hyperglycemia, core size, and inflammation.

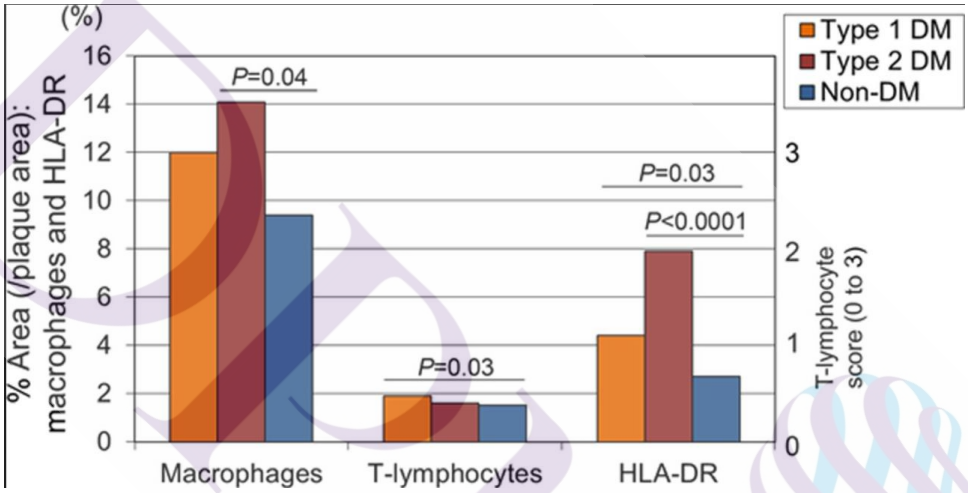


Figure 2.1 Inflammation in diabetic coronary arteries. Coronary fibroatheromas illustrating the extent of macrophages (CD68), T cells (CD45RO), and human leukocyte antigen-DR (HLA-DR) expression in patients with type 1 and 2 diabetes mellitus (DM) with control, nondiabetic subjects. **Source:** Burke et al (2004)

Burke and colleague (Burke et al, 2001) and Arenja and colleague (Arenja et al, 2013) studied healed plaque ruptures (HPR) either in the absence of symptoms or at least occurring undetected at the time of the initial event. In the context of T1DM and T2DM, there is a significantly higher incidence of asymptomatic ischemic disease, suggesting that there should be an increased frequency of HPRs in this population. The analysis used a larger number of patients of sudden coronary death registry reaffirmed the greater percentage of HPRs in subjects with T2DM relative to controls (84%

versus 55%; P=0.0008; Figure 2.2). Interestingly, 39% of sudden coronary deaths in subjects with T2D had ≥ 3 HPRs per heart when compared with only 13% of subjects without diabetes mellitus. In an earlier autopsy study involving 132 sudden death cases, they also reported a higher incidence of HPRs relative to nondiabetic controls (2.6±1.8 versus 1.9±1.8; P=0.04; Table 2.2), whereas T1DM exhibited a similar trend (2.6±2.1).7 In addition, T2DM individuals have greater plaque burden, including extensive distal vessel involvement (Table 2.2), which is consistent with the higher rates and number of HPRs per heart. This study points toward more advanced plaques and greater number of previous myocardial infarctions for those with diabetes mellitus consistent with greater atherosclerotic burden and progression relative to nondiabetics.

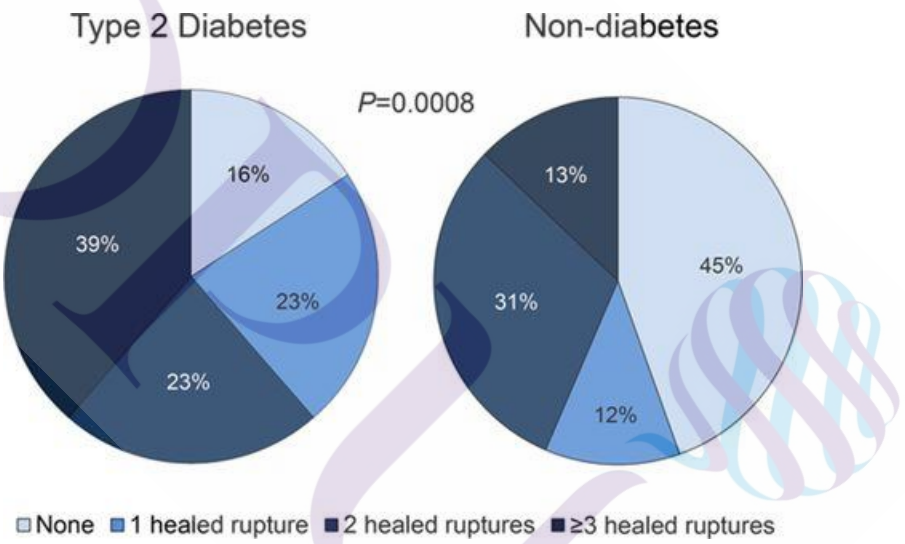


Figure 2.2 The pie charts reflect the percentage of healed ruptures (HPR) per heart relative to diabetic status at autopsy. Type 2 diabetes mellitus had higher numbers of HPRs compared with nondiabetics (*P*=0.0008). The data constitute a reanalysis of 142 sudden coronary death cases.
Source: Burke et al (2001)

Glagov and colleague (Glagov et al, 1987) studied the phenomena of coronary artery expansion referred to as positive remodeling. The results concluded that vessel enlargement is linked to increased atherosclerotic plaque burden without luminal compromise up to 40% of cross-

sectional area narrowing. It is likely that coronary plaques from patients with diabetes mellitus who survive an acute coronary event may eventually undergo negative remodeling from HPR and collagen cross-linking. However, the overall remodeling processes in patients with diabetes mellitus and coronary disease would be best confirmed by serial imaging using either noninvasive multislice cardiac computed tomography (CT) or intravascular ultrasound. On the contrary, necrotic core size and inflammation are strongly associated with internal elastic lamina expansion, particularly through proteases expressed by resident macrophages that cause extracellular matrix degradation. Therefore, one could expect greater positive remodeling considering these attributes are typically increased in diabetes mellitus.

Rumberger and colleague (Rumberger et al, 1995) studied CT coronary calcium score and atherosclerotic plaque area that shown a higher tendency for coronary artery calcification (CAC) in diabetic patients, which correlates with total plaque burden in addition to representing an independent risk factor for adverse outcomes. Other risk factors for CAC also include age and chronic kidney disease, which are inherently linked to diabetes mellitus as well. CT is the only noninvasive test with a high enough sensitivity and specificity for the detection of calcification, which has made a significant impact on the diagnosis and risk management of coronary and carotid disease. Conversely, coronary angiography has a low to moderate sensitivity compared with CT for the detection of CAC, but is specific with a high predictive value. In asymptomatic individuals with low to intermediate risk for cardiovascular events, the absence of CAC on CT is associated with very low risk (<0.5%) of obstructive noncalcified plaques on invasive angiography (Cheng et al, 2007; Blaha et al, 2016). Despite the association of CAC and future cardiovascular events, however, acute coronary syndromes may also occur in the absence of CAC, especially in young individuals who are smokers (Schmermund et al, 1997). Although the role of CAC in identifying CVD and predicting its outcome is undeniable, there is relationship with important features of diabetes mellitus such as insulin resistance and hyperglycemia is slowly evolving, especially in relation to atherosclerosis progression.

Arad and colleague (Arad, 2005) studied CAC irrespective of symptomatic or asymptomatic disease which shown the results of strongly association with future cardiac or cerebrovascular events, and the prevalence of diabetes mellitus in these individuals is high. Progressive atherosclerotic lesions (pathological intimal thickening and early fibroatheroma) with microcalcification cannot be identified by CT, whereas late obstructive coronary artery disease is strongly associated with a high CAC score (Agatston score $\geq$ 400).

Raggi and colleague (Raggi et al, 2003) investigated the prognostic value of CAC score for all-cause mortality in asymptomatic individuals including 903 patients with diabetes mellitus versus 9474 patients without diabetes mellitus after 5-year follow-up and found CAC an independent predictor of all-cause mortality independent of diabetic status. There is an established link between hyperglycemia, CAC, and diagnostic glycated HbA1c as a predictor of CVD.

Change and colleague (Change et al, 2013) revealed epidemiological evidence support of a greater risk for atherosclerotic coronary vascular disease with increasing dysglycemia, with an estimated 11% to 16% increase in cardiovascular events for every 1% increase in HbA1c. Chang et al reported greater HbA1c predicted CAC, particularly for women.

Selvian and colleague (Selvin et al, 2005) had the prospective case–cohort study of 1626 adults with diabetes mellitus showed that relative risk of coronary heart disease (CHD) was 2.37 (95% confidence interval, 1.50–3.72) for the highest quintile of HbA1c level compared with the lowest after adjustment of CHD risk factors. The risk of CHD in this study increased throughout the range of HbA1c, where in the adjusted model, the relative risk of CHD for a 1-percentage point increase in HbA1c levels was 1.14 (95 confidence interval, 1.07–1.21).

Further evidence for the predictive value of HbA1c comes from a 5-year follow-up study by Carson colleague (Carson et al, 2015) which showed that 12.9% of participants without baseline CAC developed CAC. Higher HbA1c was associated with greater incident CAC and CAC progression after adjustment for sociodemographic factors. The association of HbA1c and CAC progression persisted in multivariable-adjusted models.

Ho and Shanahan (Ho and Shanahan, 2016) described incidental micro- and macrocalcification as considered a well-known marker of subclinical atherosclerotic burden, especially in the subpopulation of individuals with diabetes mellitus. It is well-known that vascular calcification occurs in both the intima and media. However, medial calcification is rare in coronary and carotid vessels although common in medium and small muscular peripheral arteries. Both intimal and medial calcification are increased in patients with T2D, chronic kidney disease, and other less frequent disorders. In this regard, >70% of men and 50% of women with coronary artery disease with a risk factor of T1D will develop CAC by their mid-forties. The earliest coronary lesion morphology exhibiting CAC is pathological intimal thickening caused by apoptotic SMCs and matrix vesicles (MVs; 30–300 nm) appearing within cholesterol-enriched lipid pools as microcalcifications in the order of ≈ 0.5 to 15 μm in diameter. The extent of plaque calcification is exacerbated with lesion progression, as evidenced by macrophages infiltration of the lipid pool, accompanied by apoptosis/necrosis, with calcification as identified in lesions that transition to the more advanced fibroatheroma. More easily recognized are areas of confluent calcium involving the extracellular matrix and necrotic core by radiography as speckled (< 2 mm), fragmented (2–5 mm), or diffuse (≥ 5 mm; Figure 2.3) areas. The coalescence and enlargement of calcified fragments result in the formation of calcified plates/sheets, which can be visualized by noninvasive imaging modalities, such as radiography, magnetic resonance imaging, and electron-beam CT.

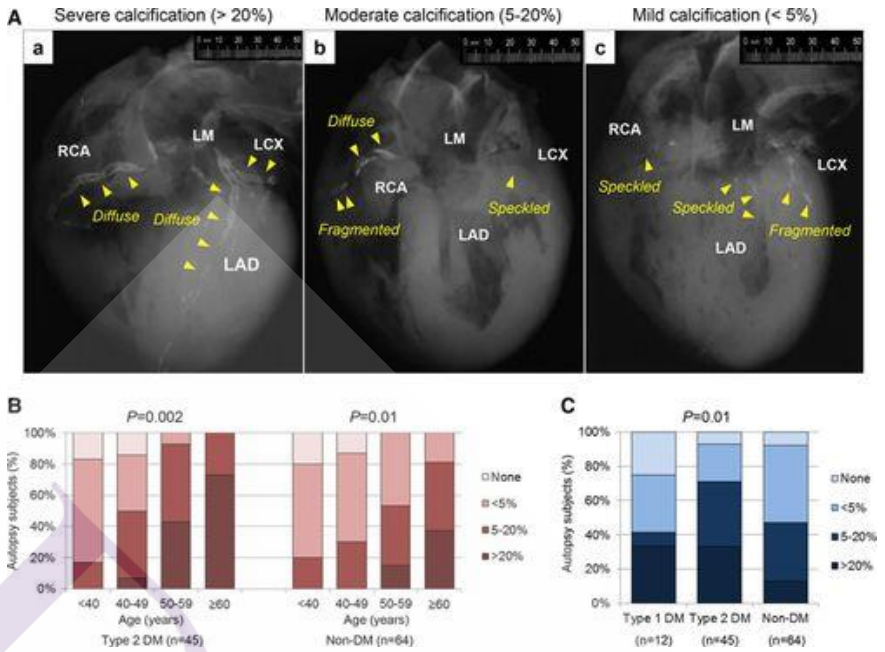


Figure 2.3 Coronary artery calcification in sudden coronary death evaluated by postmortem radiography. **A**, Representative postmortem radiographs showing various patterns of calcification. The severity of calcification was assessed based on the percentage of calcification area: (a) severe artery calcification (>20%), (b) moderate calcification (5%–20%), and (c) mild calcification (<5%). Arrows indicate type of calcification (speckled [<2 mm in length], fragmented [2–5 mm], or diffuse [≥5 mm] calcification). **B**, Percentage of total calcified area, divided into mild, moderate, and severe in sudden coronary death patients with type 2 diabetes mellitus (DM) and non-DM stratified by decade. **C**, Percentage of total calcified area in sudden coronary death comparing type 1 DM, type 2 DM, and non-DM. LAD indicates left anterior descending; LCX, left circumflex; LM, left main; and RCA, right coronary artery.

Source: Ho CY and Shanahan CM (2016)

2.3 Computed Tomography of Coronary Artery Calcium Score (CACS)

Computed tomography, more commonly known as a CT or CAT scan, is a diagnostic medical imaging test. Like traditional x-rays, it produces multiple images or pictures of the inside of the body. The cross-sectional images generated during a CT scan can be reformatted in multiple planes. They can even generate three-dimensional images. These images can be viewed on a computer monitor, printed on film or by a 3D printer, or transferred to a CD or DVD. CT images

of internal organs, bones, soft tissue and blood vessels provide greater detail than traditional x-rays, particularly of soft tissues and blood vessels.

A cardiac CT scan for coronary calcium is a non-invasive way of obtaining information about the presence, location and extent of calcified plaque in the coronary arteries—the vessels that supply oxygen-containing blood to the heart muscle. Calcified plaque results when there is a build-up of fat and other substances under the inner layer of the artery. This material can calcify which signals the presence of atherosclerosis, a disease of the vessel wall, also called coronary artery disease (CAD). People with this disease have an increased risk for heart attacks. In addition, over time, progression of plaque buildup can narrow the arteries or even close off blood flow to the heart. The result may be chest pain, sometimes called "angina," or a heart attack.

Because calcium is a marker of CAD, the amount of calcium detected on a cardiac CT scan is a helpful prognostic tool. The findings on cardiac CT are expressed as a calcium score Another name for this test is coronary artery calcium scoring.

Atherosclerosis begins in the first few decades of life with a fatty streak in which lipoproteins are deposited in the intimal and medial layers of blood vessels (Figure 2.4 and 2.5). Inflammatory cells such as macrophages and foam cells are then recruited to the areas of deposition where they cause apoptosis, creating a necrotic core with calcium deposits.

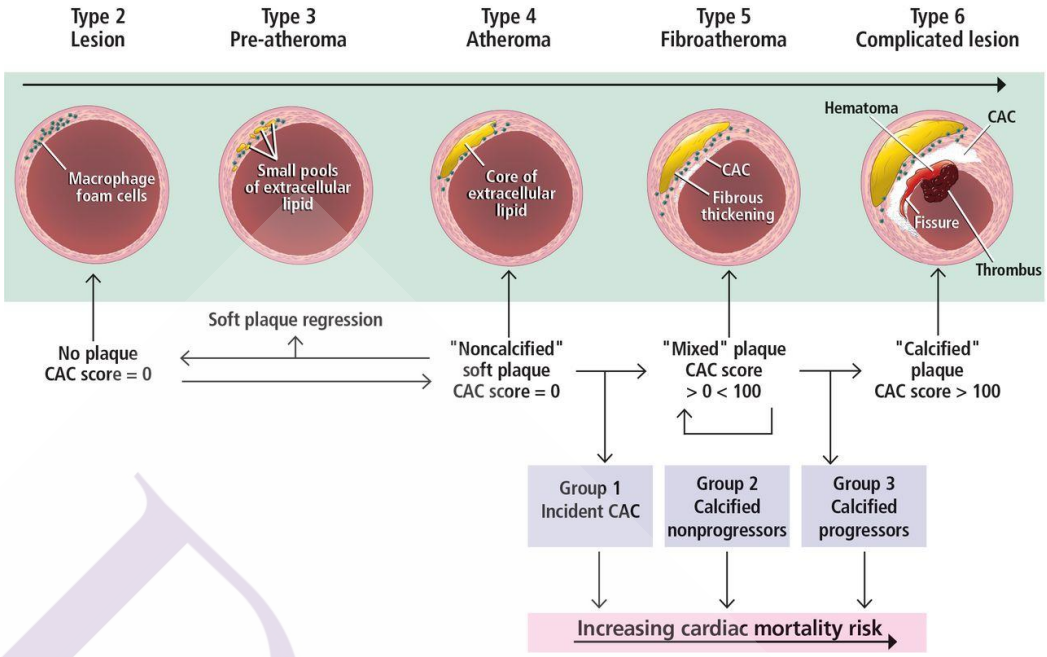


Figure 2.4 Pathogenic mechanism of atherosclerotic lesions and its relationship to the coronary artery calcium (CAC) score. A type 1 lesion (not depicted) contains lipoproteins that initiate an inflammatory response. A type 2 lesion contains an accumulation of foam cells. The type 3 lesion contains collections of extracellular lipid droplets. Eventually, these extracellular lipid pools form a lipid core, and a type 4 lesion is created. With time, this core develops a fibrous connective-tissue thickening that can calcify and give rise to a type 5 lesion detectable by imaging. Type 6 is a complicated lesion that can include thrombus from plaque rupture.

Source: Parth Parikh et al (2018)

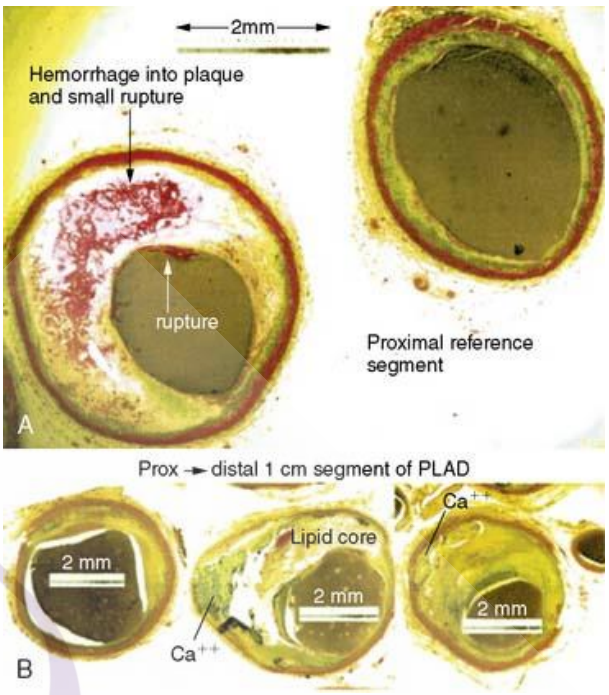


Figure 2.5 Histologic Complex plaque lesion and vessel wall remodeling. Cross-section through an atherosclerotic plaque lesion: **(A)** Overall vessel diameter is greater at the level of the lesion as a result of positive remodeling, as compared to the proximal segment, but with a much narrower lumen diameter. Lesion also indicates hemorrhage into the plaque lesion. Similarly, panel **(B)** shows changes in overall vessel diameter, luminal diameter, and degree of stenosis in a reference segment *(left)*, lipid rich plaque *(middle)*, and calcified plaque *(right)*.

Source: Burke (2002)

Fitzpatrick and colleague (Fitzpatrick et al, 1994) suggests that coronary artery calcification is an active process mediated by the production of ectopic bone matrix proteins by either vascular pericyte-like cells, smooth muscle cells or macrophage-derived foam cells. They used in situ hybridization to identify mRNA of matrix proteins associated with mineralization in coronary artery specimens. Using undecalcified sections of postmortem coronary arteries, they found mineralization to be diffuse, rather than solely confined to the intima, and present in all atherosclerotic plaques. Specifically, they identified a cell attachment protein (osteopontin), a

protein associated with calcium (osteonectin), and a γ-carboxylated protein that regulates mineralization (osteocalcin) as shown in table 2.3

Table 2.3 Biochemical Factors Implicated in Coronary Artery Calcification

Biomarker	Class	Action/Role
Osteopontin	Glycoprotein	Cell attachment
Osteonectin	Calcium-binding glycoprotein	Binds strongly to both hydroxyapatite and collagen
Osteocalcin	Gamma-carboxylated protein	Responsible for mineralization, only produced by osteoblasts
Osteoprotegerin	Cytokine of the tumor necrosis factor family (glycoprotein)	Osteoclasgenesis inhibiting the differentiation of macrophages into osteoclasts
Bone morphogenetic protein (BMP) 2a	Cytokine belonging to the transforming growth factore beta superfamily of proteins.	Induced the pericyst-like cells to undergo osteogenic differentiation leading to production of bonelike matrix

Source: Fitzpatrick et al (1994)

Coronary calcium is measured by non-contrast CT of the heart. Thus, there is no risk of contrast-induced nephropathy or allergic reactions. Images are acquired while the patient holds his or her breath for 3 to 5 seconds. Electrocardiographic gating is used to reduce motion artifact. With modern scanners, the effective radiation dose associated with calcium testing is as low as 0.5 to 1.5 mSv, about the same dose as that with mammography. The entire test takes 10 to 15 minutes.

Coronary calcium on CT is most commonly quantified using the Agatston score. Calcification is defined as a hyperattenuating lesion above the threshold of 130 Hounsfield units with an area of 3 or more pixels (1 mm^2). The score is calculated based on the area of calcification per coronary cross-section, multiplied by a factor that depends on the maximum amount of calcium in a cross-section (a weighted value system based on Hounsfield units of dense calcification in each

major coronary artery). The sum of calcium in the right coronary, left anterior descending, and left circumflex arteries gives the total Agatston calcium score.

The results fall into 5 categories, which correlate with the severity of coronary artery disease, ranging from no significant disease to severe disease (Table 2.4). Screenshot of coronary artery report and coronary artery calcium scoring images are shown on Figure 2.6 and 2.7

Table 2.4 : Coronary Artery Calcium Score (CACS) and risk of coronary artery disease (CAD)

CACS	Risk of coronary artery disease (CAD)
0	Very low risk (< 5% risk CAD)
1-10	Low risk (< 10% risk CAD)
11-100	Moderate (10 to 50% risk CAD)
101-400	High Risk (> 50% to 75% risk CAD)
Over 400	Very high rsik (>75% to 100%) risk CAD)

Source : The 2000 ACC/AHA Expert Consensus document

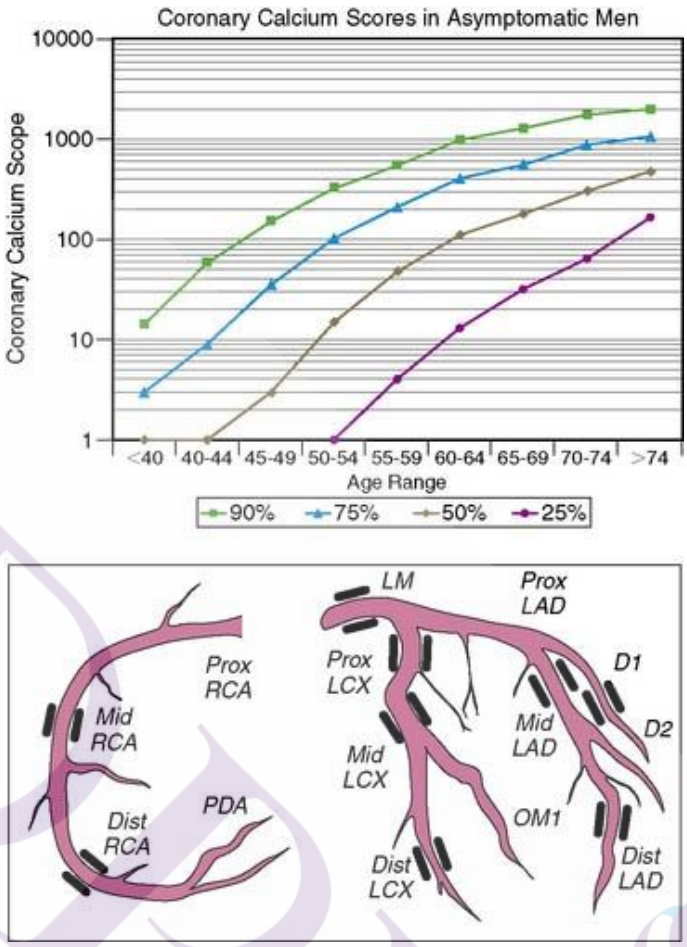


Figure 2.6 Typical coronary artery calcification (CAC) report. Upper image**,** The CAC scores obtained are plotted on this age- and sex-specific nomogram, with CAC scores divided by quartiles. The *red star* represents the percentile rank for this particular patient. This example has been generated by the TeraRecon (San Mateo, California) workstation. Lower image**,** Diagrammatic representation of distribution of calcium in the coronary arteries.

Source : TeraRecon (San Mateo, California) workstation (2000)

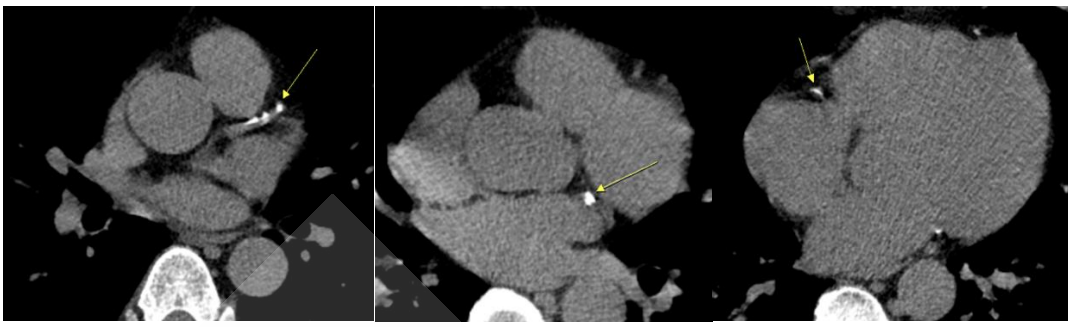


Figure A: axial CT LAD **Figure B:** axial CT LCx **Figure C:** axial CT RCA

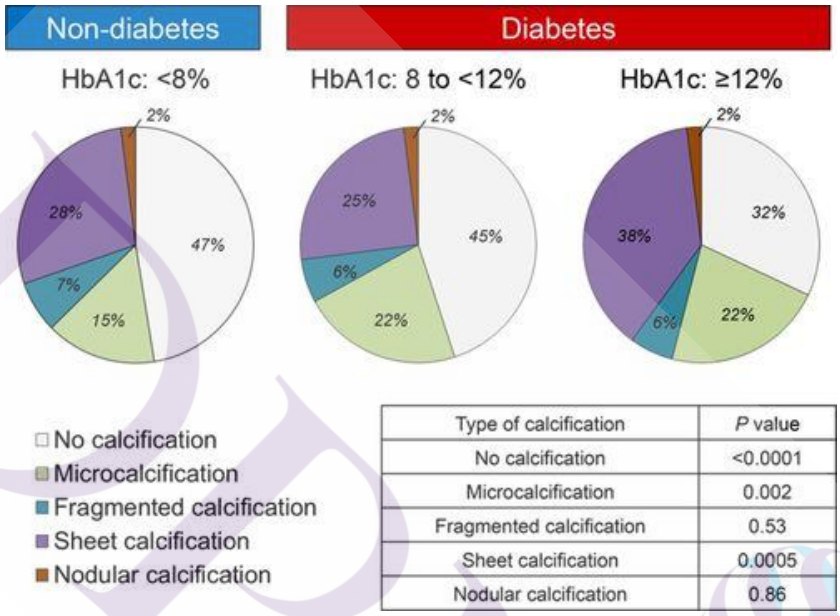
Figure 2.7 Axial CT scan coronary artery calcium score (CACS) of a 58-year old male; Figure A showed calcified proximal segment of left anterior descending artery (LAD); Figure B showed calcified proximal left circumflex artery (LCx); Figure C showed calcified proximal segment of right coronary artery (RCA)

Source: CT Images of Kasemrad International Hospital, Thailand (2020)

Recommendation of asymptomatic patients suitable for CAC is the aged 45-75 years with intermediate cardiovascular risk (10-20%). There is a possible role for CAC in those aged 45-75 years with lower cardiovascular risk (6-10%) as defined by FRS in: (1) Those with a strong family history of premature CHD, (2) Diabetics aged 40 –60 years old, and (3) Indigenous patients (Aboriginals, Maori and Pacific Island patients) >40 years old.

Burke and colleague (Burke et al, 2007) exhibited the appearance of sheets integrated within fibrotic tissue consisting of collagen and SMCs, which may or may not involve the necrotic core. Nodular type of calcification is the least common form existing as small calcified fragments with interspersed fibrin. In the context of lesion progression, the extent of CAC has been shown to correlate with plaque burden, but not necessarily with the severity of luminal narrowing. In their review of postmortem radiographs of sudden death hearts, the percentage of CAC (based on percentage of calcification area) was evaluated as absent, mild (<5%), moderate (5%–20%), or severe (>20%). Although the percentage of CAC relative to plaque burden increased progressively over decades for T2D (P=0.002) and in controls without diabetes mellitus (P=0.01), lesions from

subjects with either T1D or T2D exhibited an increase in areas of severe CAC (*P*=0.01). When sudden death cases were stratified by HbA1c, there was a decline in the number of lesions without calcification and significant reciprocal increase in sheet calcification with escalating HbA1c levels in patients with diabetes mellitus (Figure 2.8)



Type of calcification	*P* value
No calcification	<0.0001
Microcalcification	0.002
Fragmented calcification	0.53
Sheet calcification	0.0005
Nodular calcification	0.86

Figure 2.8 Percentage of sudden deaths based on coronary artery calcification type (none, micro, fragmented, sheet, and nodular), stratified by HbA1c level (<8%) nondiabetics and diabetic (8% to <12% and ≥12%). Histological sections (1630; <8%, n=776 sections; 8% to <12%, n=548; and ≥12%, n=306) from 57 patients with stable coronary artery disease were examined. Note the declining shift in the number of lesions without calcification and significant reciprocal increase sheet calcification with escalating HbA1C levels.

Source: Burke et al (2007)

Odink and colleague (Odink et al, 2010) studied 1002 patients aged ≥ 55 years assessed calcification on multislice cardiac CT in the coronary, aortic arch, and carotid arteries and showed that diabetes mellitus was an independent risk factor for CAC in men and women, whereas in the carotid territory, correlations were selective only for women. Patients with T2D undergoing dental panoramic radiography had a 5-fold excess prevalence of calcified carotid arteries, as compared with patient without diabetes mellitus. Similar to findings in coronary bed, HbA1c has also been shown to be an independent predictor of stroke and CVD in both individuals with and without diabetes mellitus.

Spagnoli and colleague (Spagnoli et al, 1994) examined 180 carotid endarterectomy specimens from patients presenting with transient ischemic attack or stroke and showed that fibrous plaques correlated with both aging and diabetes mellitus. On the contrary, the presence of thrombosis was significantly less in patients with diabetes mellitus than in those without diabetes mellitus (19.6% versus 42.0%), which is in agreement with several literature reviewed about coronary arteries.

In another study by Scholtes and colleague (Scholtes et al, 2014) studied patients with T2D (295 patients), no differences were observed in carotid plaque morphology or expression of inflammatory chemokines, cytokines, or advanced glycation end products (AGEs) between T2D and nondiabetics. The study results may be negative because T2D had a higher proportion of previous cardiovascular interventions along with more stringent treatment for hypertension and hypercholesterolemia compared with patients without diabetes mellitus (1160 patients).

Clinical studies of Vigili and colleague (Vigili et al 2015) demonstrated the value of carotid plaque calcification for discriminating future cardiovascular events and death in patients with T2D where the risk of MACE (major adverse cardiovascular events) progressively increases with plaque calcification.

In MESA study (Multi-Ethnic Study of Atherosclerosis), 946 participants were evaluated by magnetic resonance imaging and ultrasound to determine remodeling index and carotid artery thickness (Zavodni et al, 2014). The event rate including myocardial infarction, resuscitated cardiac arrest, angina, stroke, and death was significantly greater in diabetics than in nondiabetics. In addition, calcification was also greater in diabetics along with remodeling index and carotid thickness.

In a large magnetic resonance imaging plaque imaging study of Esposito and colleague Esposito et al, 2010), there were 191 patients, moderate- to high-grade carotid artery stenoses and advanced lesion phenotypes, of T2DM patients (57%), and multiple logistic regression analysis revealed association between T2D and magnetic resonance imaging–defined high-risk lesion types (odds ratio, 2.59; 95% confidence interval, 1.15–5.81), independent of the degree of stenosis.

2.4 Patterns and Genetics of Vascular Calcification

Demer and Tintut were divided into 3 types of vascular calcification; (1) inflammatory, (2) metabolic, and (3) genetic, with the latter being mostly medial. Contrary to medial calcification which is rarely observed in coronary or carotid vessels, intimal calcification is a systemic process influenced by traditional CVD risk factors in addition to local effects of oxidative stress and inflammation. Despite the association between aging and calcification, there is a strong correlation of coronary calcium score with CHD independent of traditional risk factors. This study showed that the adjusted risk of a coronary event was significantly increased by a factor of 7.73 among individuals with CAC score between 101 and 300 and by a factor of 9.67 with CAC >300 when compared with those with no CAC, with event rates being significantly higher in diabetes mellitus. Therefore, coronary calcification provides predictive information beyond that provided by standard risk factor (Demer and Tintut, 2014).

On the contrary, Pugliese and colleague (Pugliese et al, 2014) presented the dynamics of vascular calcification in the context of coronary disease progression is far more complex. It is well recognized that calcification has a dual function, which either promotes plaque progression toward an unstable, rupture-prone phenotype or favors stabilization, depending on the type and pattern of calcium deposition. In general, the patterns of vascular calcification are clinically meaningful such that spotty or granular microcalcification is associated with a proinflammatory process and lesion instability while sheet-like or laminated macro-calcification is often observed in fibrotic lesions and is thought to support plaque stabilization.

Evrard and colleague (Evrard et al, 2015) studied biochemical factors, there is also a strong genetic predisposition where genome-wide association studies have identified multiple contributing loci (6p21.3, 6p24, 10q21.3, and 9p21; Figure 2.9) linked to atherosclerosis, diabetes mellitus, and coronary calcification. Methodological limitations, however, present an issue in assessing types of vascular calcification, the inability to separate genetic processes underlying intimal from medial calcification although there is strong experimental and clinical evidence that argues for the continued distinction between mechanisms underlying intimal and medial calcification. Moreover, because plaque burden and CAC are well correlated, genetic associations could also be confounded by genes underlying atherosclerotic disease rather those controlling calcification.

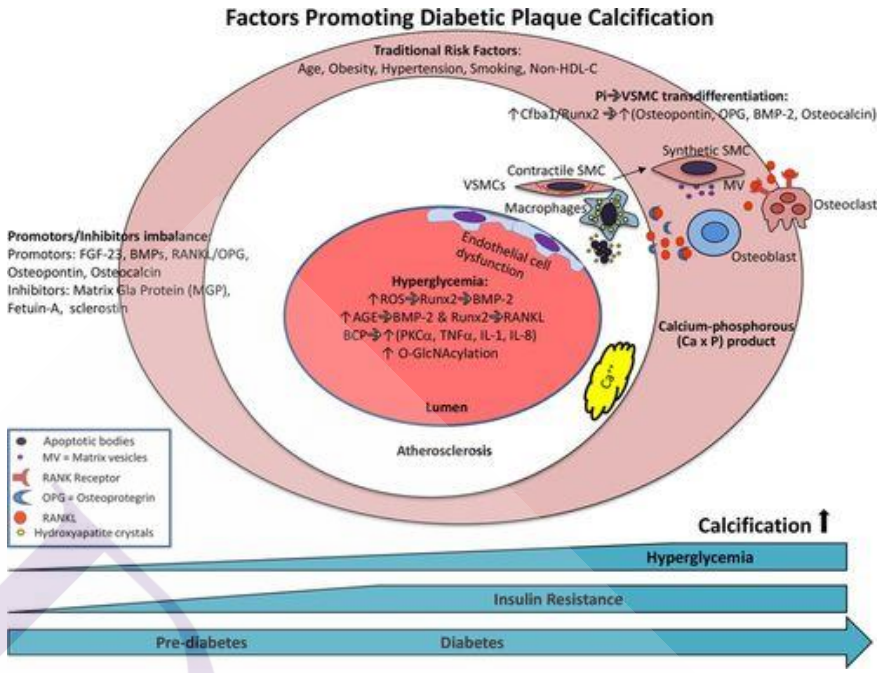


Figure 2.9 Mechanisms of plaque calcification in diabetes mellitus. The earliest form of calcification, microcalcification occurs in apoptotic vascular smooth muscle cells (VSMCs) and macrophages in conjunction with an increase in serum calcium-phosphorous (CaxP) product. Increases in phosphate concentration in VSMCs induce a switch toward an osteoblast-like phenotype, particularly in states of P_i excess. Osteogenic-primed VSMCs express alkaline phosphatase (ALP) and under the control of Cfba-1, secrete bone-associated proteins such as osteopontin, collagen type 1, osteoprotegerin, BMP (bone morphogenic protein)-2, and osteocalcin, accompanied by the release of mineralization-competent matrix vesicles (MVs). Changes in promoters or inhibitors of mineralization also affect calcification. Hyperglycemia may also affect calcification through multiple mechanisms such as oxidative stress, advanced glycation end products (AGEs), basic calcium phosphate (BCP), O-linked β-N-acetylglucosamine modification (O-GlcNAcylation), and endothelial dysfunction as discussed in the text. EPCs indicates endothelial progenitor cells; FGF, fibroblast growth factor; HDL-C, high-density lipoprotein-cholesterol; IL, interleukin; MCCs, myeloid calcifying cells; MV, matrix vesicle; OPG, osteoprotegrin; ROC, reactive oxygen series; Runx2, Runt-related transcription factor-2; TGF, transforming growth factor; and TNF, tumor necrosis factor.

Source: Evrard (2015)

Despite its clinical importance, the molecular complexities involved in the regulation of vascular calcification remain incompletely understood. It is worth mentioning for understanding of the genetic basis for vascular calcification has been advanced through mouse models but in general arterial calcification and atherosclerosis do not occur together in these models (unlike in humans). It is rare to see arterial calcification in atherosclerosis-prone mice (ie, apolipoprotein E or low-density lipoprotein receptor knockouts). Similarly, mice deficient in osteoprotegerin or matrix GLA (γ-carboxyglutamate) protein (reviewed below) demonstrate arterial medial calcification in the absence of atherosclerosis.

Thus, human lesions are unique because of the cosegregation calcification with atherosclerosis even in the earliest atherosclerotic lesion (ie, pathological intimal thickening), which is not completely replicated by any available animal models, with the exception of the monkey. Although at least 4 different nonmutual exclusive mechanisms of vascular calcification have been proposed, some are more relevant to the process of medial rather than intimal calcification. The process of medial vascular calcification is viewed as a hydroxyapatite mineralization process within the medial layer. Loss of inhibitors of mineralization such as MGP (matrix Gla protein) and pyrophosphate promote medial calcification in mice. Bone formation inside the vessel wall is also rarely observed in human vascular lesions and suggests that osteogenic mechanisms may also play a role in vascular calcification. Bone-forming proteins such as osteopontin, collagen type 1, osteoprotegerin, BMP-2 (bone morphogenic protein-2), and osteocalcin and release of mineralization-competent MVs are critical in this process.

Vascular SMCs (VSMC) can undergo osteogenic transformation into phenotypically distinct osteoblast-like cells that are capable of expressing and releasing osteochondrogenic proteins. Such changes have rarely been observed in vivo in humans. Cell death can also provide phospholipid-rich debris that serve to nucleate apatite. This process starts within lipid pools and progresses with inflammation and the development of necrotic core as lesions process.

Finally, elevated Ca or phosphorus (P) promotes apatite nucleation and crystal growth that further promotes vascular calcification via what are referred to as thermodynamic mechanisms. Such mineral imbalances may also exacerbate processes initiated by the other mechanisms mentioned above. (Luo et al, 1997; Zhou et al, 2012; Jornson, 2006; Harper et al, 2016)

Schinke and colleague (Schinke et al, 2007) studied physiological bone formation and ectopic calcification that are often viewed as active processes with the expression of a mineralizing extracellular matrix partially under hormonal control, whereas diminished activity or loss of calcification inhibitors also leads to arterial calcification but is viewed as a passive process. Many of the hormonal and physiological abnormalities associated with diabetes mellitus can promote intimal calcification, including oxidative stress, endothelial dysfunction, alternations in mineral metabolism, increased inflammatory cytokine production, and release of osteoprogenitor cells from the marrow into the circulation. Below we briefly discuss some of these mechanisms as they relate to the development of vascular calcification in patients with diabetes mellitus.

2.6 Hyperglycemic Mechanisms related Vascular Calcification

The research of Nishikawa and colleague (Nishikawa et at, 2000) is the one of the most significant discoveries in patients with diabetes mellitus that unraveling the nature of hyperglycemic damage mainly driven by the accumulation of free radicals, namely, superoxide anion, which is capable of activating an array of cellular pathways including polyol and hexosamine flux, advanced glycation end products (AGEs), PKC, and nuclear factor-κB–mediated vascular inflammation. The results shown increased levels of glucose and other reducing sugars such as galactose and fructose reacted amino groups of proteins to form Schiff bases to yield AGEs. The interaction of AGEs with receptors for advanced glycation end production (RAGEs) activates PKC-ζ to trigger downstream activation of signaling through p38 mitogen activated PK, transforming growth factor-β, and nuclear factor-κB. Significant data support that AGE treatment of VSMC promotes calcification through multiple mechanisms including increasing levels of alkaline phosphatase (ALP), a bone matrix protein, decreased expression of VSMCs markers, and increased

expression of Runt-related transcription factor-2 (Runx2), suggesting RAGE promotes transformation of VSMCs into osteoblast-like phenotype.

Kay, Simpson and Stewart (2016) studies AGE/RAGE signaling, the results showed AGE/RAGE signaling exacerbates oxidative stress though a feed-forward loop. AGE activation results in the increased production of reactive oxygen species by stimulating specific signaling cascades such as transforming growth factor-β, nuclear factor-κB, and Nox-1. SMC expression of S100A12, a human RAGE ligand, increased medial calcification in proximal aorta and innominate arteries of ApoE mice, which was associated with increases in BMP-2 and Runx2.
The actions of S100A12 were dependent on RAGE and oxidative stress signaling because both recombinant soluble RAGE (decoy receptor for RAGE) and NAD(P)H oxidase (Nox) inhibition reduced osteogenic programming and calcification. Ligands for RAGE are quenched by the soluble form of the receptor, and serum levels of this decoy receptor in hemodialysis patients are inversely associated with vascular calcification. In each of these experiments, although AGEs have been shown to induce vascular calcification, the specific links to diabetes mellitus remain somewhat elusive.

Hyperglycemia itself also increases oxidative stress by increasing glucose oxidation in the citric acid cycle. Generation of mitochondrial reactive oxygen species (ROS) is an important contributing factor, as treatment with a mitochondrial uncoupler prevents upregulation of reactive oxygen species. When glucose levels are elevated, the polyol pathway converts glucose to sorbitol using NADPH (nicotinamide adenine dinucleotide phosphate hydrogen) as a cofactor. As a result, the antioxidant glutathione, which also uses NADPH as a cofactor, becomes dysfunctional decreasing cellular resistance to oxidative stress. Many groups have shown that oxidative stress powerfully upregulates Runx2 and promotes VSMC calcification. Oxidative stress and oxidized lipids also induce receptor activator of nuclear factor-κB ligand in mouse VSMCs via Runx2. Mice deficient in a decoy receptor for receptor activator of nuclear factor-κB ligand, osteoprotegerin, develop extensive vascular calcification which is reduced by osteoprotegerin treatment (Bucay et al, 1998; Panizo et al, 2009).

Hyperglycemia also can activate the PKC pathway by increasing the synthesis of diacylglycerol that plays a critical role in activating PKC, PKCβ, PKCδ, and PKCα. Globally, genes involved in vessel dilation such as nitric oxide are decreased, whereas those involved in vessel constriction such as endothelin-1 are increased. Basic calcium phosphate crystals deposit in atherosclerotic lesions and colocalize with inflammatory macrophages.

Nadra and colleague (Nadra et al, 2005) showed that ingestion of basic calcium phosphate in macrophages triggers a proinflammatory response, including secretion of the inflammatory cytokines tumor necrosis factor-α, interleukin-1, and interleukin-8. PKCα was a key mediator of these effects. Others have suggested tumor necrosis factor–induced nuclear factor-κB can promote inorganic phosphate-induced calcification of human aortic SMCs while suppressing pyrophosphate (inhibitor of calcification). Thus, activation of PKC by hyperglycemia might produce a vicious cycle whereby ingestion of basic calcium phosphates by macrophages not only induces inflammation but also promotes calcification.

Glucose metabolism through the hexosamine biosynthetic pathway produces UDP-β-d-N-acetylglucosamine, an active sugar donor for O-linked β-N-acetylglucosamine modification (O-GlcNAcylation). (Nagel et al, 2013). O-GlcNAcylation is the glycosylation process through which N-acetylglucosamine (O-GlcNAc) gets added to serine and threonine residues of proteins. O-GlcNAcylation has been shown to stimulate chondrogenesis and osteogenesis and correlates with the transcriptional activity of the osteogenesis regulator, Runx2.

Heath and colleague (Heath et al, 2014) identified O-GlcNAcylation of AKT on T430 and T479 amino acids as a potential regulator of diabetes mellitus–induced calcification. In streptozotocin-treated mice, they found a strong increase in vascular O-GlcNAcylation along with increases in vascular calcification. Blocking the removal of O-GlcNAc further enhanced calcium levels both in vitro in cultured VSMCs and in vivo in mice.

Shao and colleague (Shao et al, 2005) studied endothelial cell dysfunction in diabetic patients. Major risk factors for endothelial cell dysfunction in T1D are poor glycemic control and diabetes mellitus duration, whereas in T2D, insulin resistance is an important risk factor. The mechanisms by which hyperglycemia induces the activation of various pathways (DAG, PKC, and hexosamine) of glucose metabolism and production of oxidative stress, formation of AGEs, etc, also apply to endothelial cells. Driven by hyperglycemia and oxidative stress, apoptosis of endothelial cells and their overall dysfunction may promote endothelial permeability, exposing VSMCs to hyperglycemia and other proinflammatory circulating factors known to promote calcification such as ALP and receptor activator of nuclear factor-κB ligand. Moreover, production of tumor necrosis factor-α from both endothelial and SMCs induces production of BMP-2, a potent osteoblastic differentiation factor, which promotes osteogenenesis by activating the homeobox homolog (Msx2) and Wnt signaling pathways.

2.7 Dysregulation of Phosphate Homeostasis and Vascular Calcification: The Bone–Kidney–Vascular Axis

Block and Port (Block and Port, 2000) showed serum phosphate levels link with a tendency toward vascular calcification, as high serum phosphate levels (hyperphosphatemia, ie, phosphate levels higher than the normal adult range of 1.0–1.5 mmol/L) highly correlate with the extent of vascular calcification and vascular disease. One of the most common causes of hyperphosphatemia is chronic renal failure treated with hemodialysis, in which serum inorganic phosphate (P_i) levels can typically exceed 2 mmol/L and are commonly associated with widespread vascular calcification. Vascular calcification observed in these patients is routinely referred to as metastatic calcification because it occurs in the presence of a systemic mineral imbalance.

High levels of phosphate may exacerbate mechanisms of vascular calcification mediated by osteogenic transcription factors. VSMCs can undergo osteogenic transformation into phenotypically distinct osteoblast-like cells that are capable of expressing and releasing osteochondrogenic proteins. Increases in phosphate concentration in VSMCs induce a switch toward an osteoblast-like phenotype mainly driven by Cfba1/Runx2 (core-binding factor subunit 1α/runt-related transcription factor 2), particularly in states of P_i excess. Osteogenic-primed

VSMCs express ALP and under the control of Cfba-1 secrete bone-associated proteins such as osteopontin, collagen type 1, osteoprotegerin, BMP-2, and osteocalcin accompanied by the release of mineralization-competent MVs.

Levy and colleague (Levy et al, 1983) revealed approximately 50% of diabetic subjects develop microalbuminuria, which progresses toward established diabetic nephropathy in one third of patients and elevated serum phosphate. Phosphate homeostasis is maintained by the gut, bone, and kidney and is regulated by many hormones such as parathyroid hormone, and 1α,25-dihydroxyvitamin D3 (1α,25-(OH)2D3), and the more recently described fibroblast growth factor (FGF) 23 and its required cofactor Klotho. About the latter, FGF23 is a circulating phosphaturic hormone that is elevated in patients with chronic kidney disease and strongly associated with cardiovascular mortality. High plasma FGF23 concentrations independent from traditional risk factors have also been observed in blacks and patients with T2D mellitus and high CAC scores.

Freedman and colleague (Freedman et al, 2015) described the specific link between FGF23 and vascular calcification, however, is unclear, considering FGF23 or its coreceptor, klotho, does not seems to be present in human or mouse VSMCs or normal or calcified mouse aorta. Moreover, quantified coronary artery and thoracic aortic calcification by CT in 1501 patients from the CRIC study (Chronic Renal Insufficiency Cohort) showed that baseline plasma FGF23 was not associated with the prevalence or severity of calcification even after multivariable adjustment. The absence of FGF23/klotho expression in both mouse and human SMCs combined with the failure to show a direct association with vascular calcification possibly argues that this pathway may not have a direct effect on vascular calcification.

The initiation of calcium phosphate deposits may start as MVs, which are then released by mineralization-competent cells into extracellular matrix. MVs are spherical bodies in 30 to 300 nm in diameter, enriched in tissue-nonspecific ALP, which is indispensable for mineralization. It has long been established that inorganic pyrophosphate or polyphosphate must be removed from the sites of mineralization, before calcification can occur. VSMCs release MVs under normal physiological conditions, and these MVs are protected from mineralization by the presence of calcification inhibitors. Under pathological conditions, however, a combination of factors,

including the influence of tissue-nonspecific ALP, makes the MVs mineralization competent. (Anderson 2003; Whyte, 2010, Hessle et al, 2002).

2.8 Circulating Cell Theory of Vascular Calcification

An emerging theory (Speer et al, 2009) suggested that VSMCs may also transdifferentiate to form osteochondrogenic precursors. This process is characterized by a phenotypic change in the expression of the osteochondrogenic markers osteopontin, osteocalcin, and ALP in addition to the osteochondrogenic transcription factors core binding factor a1 and Runx2 with a reciprocal loss in the VSMC marker α-smooth muscle actin. Two recent studies found that the proportion of circulating progenitor cells with osteogenic markers is significantly increased in patients with diabetes mellitus. The exact role of these cells in the setting of diabetic calcification is still being investigated.

In addition, a subpopulation of circulating myeloid-derived calcifying cells has been identified and suggested to be involved in vascular calcification, especially in subjects with T2DM, which is characterized by increased circulating levels of these cells, which express ALP and Runx2. The transdifferentiation of VSMCs toward an osteochondrogenic phenotype may participate in the initial phase of calcification; it especially characterizes the subsequent, eventual formation of large plates of organized calcium deposits (ie, macrocalcification) within the vessel wall, which may even recapitulate mature bone tissue.

2.9 Clinical Studies of CVD and Bone Matrix Regulator Proteins

Clinical study of Zhang and colleague (Zhange et al, 2015) had reported the association between coronary artery disease and bone matrix regulatory proteins. For instance, plasma BMP-2 level was significantly higher in T2DM patients than in non diabetes mellitus patients. In a multivariable linear regression analysis, plasma BMP-2 was significantly and positively associated with HbA1c and Syntax score. Interestingly, plasma BMP-2 level was positively correlated with plaque burden and CAC assessed by intravascular ultrasound, whereas negatively correlated with lumen volume.

Similarly, Chen and colleague (Chen et al, 2006) showed that high serum glucose levels were associated with an increased expression of core binding factor a1 and BMP-2, which enhanced the calcification of VSMCs.

2.10 Cardiovascular health and cardiovascular disease

Cardiovascular health (CVH) is defined by the absence of clinical manifestation of CVD together with the presence of optimal levels of all life's Simple 7 (LS7). These include 4 health behaviors (not smoking, healthy diet pattern, sufficient physical activity, normal body weight), and 3 health factors (normal level of total cholesterol, of blood pressure, and fasting blood glucose) in the absence of drugs treatment (AHA, 2016). A meta-analyze of 9 prospective cohort studies among 12,878 persons reported that ideal cardiovascular health metrics was associated with lower risk of all-cause mortality (Fang, Jiang & Fan, 2016). The Reason for Geographic and Racial Difference in Stroke (REGARDS) cohort among 22,914 subjects with LS7metrics data and no previous cardiovascular disease showed that every better health category of the LS7 score was associated with 25% decrease in the risk of stroke (Kulshreshtha et al., 2013). Better cardiovascular health is associated with decrease risk of vascular disease (Saleem et al., 2015). Yang et al examined the association between LS7metrics and CVD mortality over a 14.5 years period; individuals with ≥ 6 healthy factors, had an absolute CVD mortality risk of 15% compared to 65% for those reporting only one or no healthy behaviors (Yang et al., 2012).

2.10.1. Cardiovascular disease definitions

CVDs, refers to various chronic pathology or events that have in common a pathophysiology related to atherosclerosis and including:

• Coronary artery disease (CAD): stable angina, unstable angina, myocardial infraction, sudden death

• Cerebral vascular accident: stroke: hemorrhagic, ischemic or transit ischemic attack

• Peripheral Artery Disease (PAD): Lower Extremity Artery Disease (LEAD), aortic aneurysm

• Congestive heart failure (CHF)

2.10.2. Types of cardiovascular disease

2.10.2.1 Coronary artery disease

Definition and classification Also, known as ischemic heart disease (IHD) refers to conditions that involve impairment of coronary artery blood flow that can result in silent ischemia, angina pectoris, acute coronary syndrome (ACS) or sudden cardiac death. Coronary artery disease (CAD) is a common public health problem associated with high mortality and increased health cost (He et al., 2017).

Physiopathology

Atherosclerosis is a complex progressive chronic multifocal, immune-inflammatory, fibro proliferative disease, with the accumulation of lipid metabolism, active cellular interaction, inflammation and matrix remodeling in the large arteries (Brown et al., 2017; Hamm et al., 2006). Atherosclerosis causes complex, lesions on coronary, cerebrovascular and peripheral vascular diseases (Tabas, García-Cardeña & Owens, 2015). The anatomy of normal artery is displayed in Figure 2. The early lesion of atherosclerosis consists of fatty streak comprised of (cholesterol and macrophage). They are limited to the aorta in the first decade of life, then extend later to the coronary arteries and peripheral arteries. The lesion leads to acute occlusion of the artery by a thrombus often related to intimal of rupture or erosion, clinically present as unstable angina, Myocardial Infarction (MI) or sudden cardiac death (Lusis, 2000, Anon, 2017a). In Stable Coronary

Artery Disease (SCAD), atherosclerosis lesion progresses slowly, allowing for the development of collateral circulation (T. Wang and Butany 2017) (figure 3). The atherosclerosis process can be accelerated by the cardiovascular traditional risk factors such as diabetes, hypertension, obesity, dyslipidemia, smoking, and genetics factors.

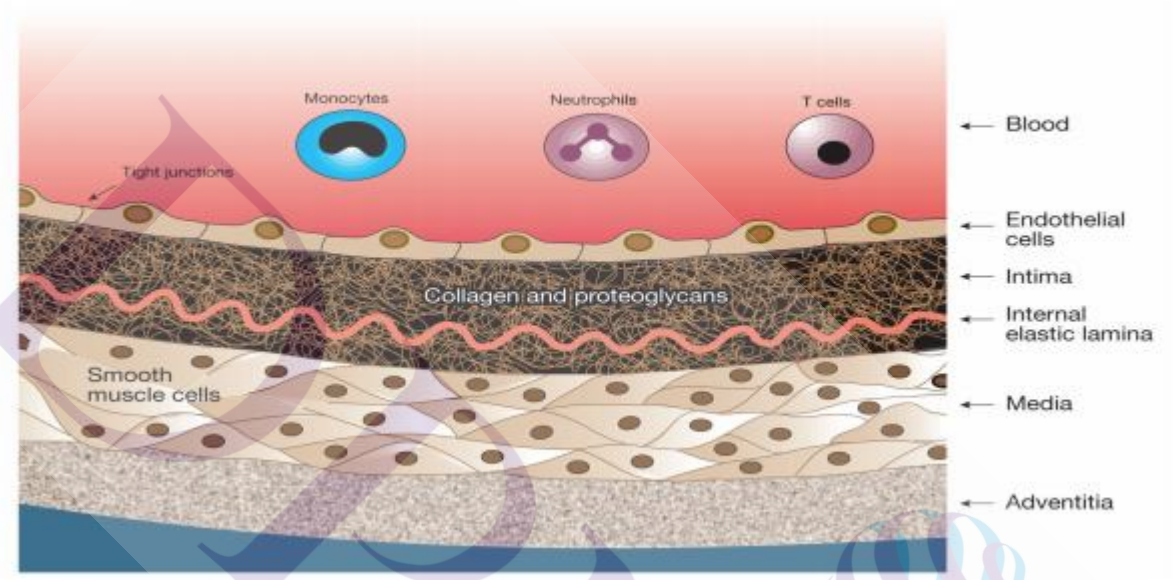


Figure 2.10 Structures of a normal large artery

Source: Lusis (2000)

A large artery consists of three morphologically distinct layers. The intima, the innermost layer, is bounded by a monolayer of endothelial cells on the luminal side and a sheet of elastic fibres, the internal elastic lamina, on the peripheral side. The normal intima is a very thin region (size exaggerated in this figure) and consists of extracellular connective tissue matrix, primarily proteoglycans and collagen. The media, the middle layer, consists of SMCs. The adventitia, the outer layer, consists of connective tissues with interspersed fibroblasts and SMCs.

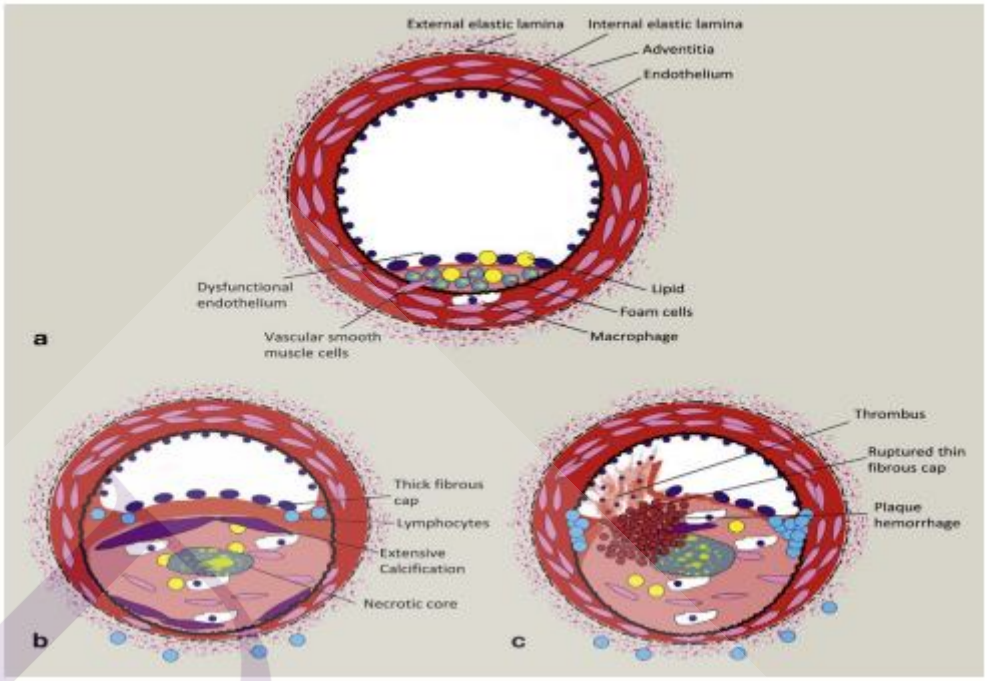


Figure 2.11: (a) Fatty streak with dysfunctional endothelial cells, lipid insudation and the macrophage transformation into foam cells. (b) Stable plaque with extensive calcification and thick fibrous cap overlying foam cells and necrotic core. (c) Acutely ruptured unstable plaque, with a collection of fibrin and platelets forming a thrombus over the disrupted thin fibrous cap.

Source: Wang & Butany (2017)

2.10.2.2 Acute coronary syndrome

Acute coronary syndrome refers to a spectrum of clinical symptoms compatible with acute myocardial ischemia and includes Unstable Angina (UA), Non-ST Segment Elevation Myocardial Infarction (NSTEMI), and ST-segment Elevation Myocardial Infraction (STEMI), thus they share common pathophysiological origins related to coronary plaque progression, instability, or rupture with or without luminal thrombosis and vasospasm. The main clinical expression are MI, and sudden cardiac death. Differentiating ACS from other cardiac chest pain is the primary diagnostic challenge. The initial assessment requires a good history collection including risk factors analysis, a physical examination, an Electrocardiogram (ECG) and cardiac biomarkers analysis that help in

determining the differential diagnosis such as aortic dissection, pericarditis, pulmonary embolism and musculoskeletal pain (Braunwald et al., 1994).

Clinical presentation of Acute coronary syndrome Most patients describe diffuse severe pain, which may occur with exertion or at rest localized in the sub sternal region in typical cases or epigastric discomfort. The pain radiates to the neck, jaw, left shoulder and left arm or both, not affected by movements. Other symptoms can be associated such as nausea, vomiting, unexplained fatigue and in rare cases syncope. Discomfort persists more >20 Minutes (min). Symptoms might be atypical in diabetic patients, women and elderly persons (Kumar & Cannon, 2009; Thygesen et al., 2012). Five factors reinforce the diagnosis of acute ischemia due to CAD. They are according their weight: a past history of CAD, male sex, older age, the characteristics of angina pain and the presence of cardiovascular risk factors such as HTN, DM, dyslipidemia, cigarette smoking, and family history of premature CAD (Pryor et al., 1993).

2.10.2.3 Myocardial infraction

Acute myocardial infarction is a myocardial necrosis due to prolonged ischemia (Thygesen et al., 2012). The diagnosis is based on biochemical criteria (troponin elevation as a result of irreversible cell damage), clinical evaluation, ECG findings, invasive and noninvasive imaging and pathological evaluation (Table 2.5) (Anderson & Morrow, 2017).

Table 2.5: New Universal classification of myocardial infarction

Type 1: Spontaneous myocardial infarction
Spontaneous myocardial infarction related to atherosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection with resulting intraluminal thrombus in one or more of the coronary arteries leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis. The patient may have underlying severe CAD but on occasion non-obstructive or no CAD.
Type 2: Myocardial infarction secondary to an ischaemic imbalance
In instances of myocardial injury with necrosis where a condition other than CAD contributes to an imbalance between myocardial oxygen supply and/or demand, e.g. coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachy-/brady-arrhythmias, anemia, respiratory failure, hypotension, and hypertension with or without LVH.
Type 3: Myocardial infarction resulting in death when biomarker values are unavailable
Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurring before blood samples could be obtained, before cardiac biomarker could rise, or in rare cases cardiac biomarkers were not collected.
Type 4a: Myocardial infarction related to percutaneous coronary intervention (PCI)
Myocardial infarction associated with PCI is arbitrarily defined by elevation of cTn values >5 x 99^{th} percentile URL in patients with normal baseline values (£99^{th} percentile URL) or a rise of cTn values >20% if the baseline values are elevated and are stable or falling. In addition, either (i) symptoms suggestive of myocardial ischemia, or (ii) new ischemic ECG changes or new LBBB, or (iii) angiographic loss of patency of a major coronary artery or a side branch or persistent slow- or no-flow or embolization, or (iv) imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality are required.
Type 4b: Myocardial infarction related to stent thrombosis
Myocardial infarction associated with stent thrombosis is detected by coronary angiography or autopsy in the setting of myocardial ischemia and with a rise and/ or fall of cardiac biomarkers values with at least one value above the 99^{th} percentile URL.
Type 5: Myocardial infarction related to coronary artery bypass grafting (CABG)
Myocardial infarction associated with CABG is arbitrarily defined by elevation of cardiac biomarker values >10 x 99^{th} percentile URL in patients with normal baseline cTn values (£99^{th} percentile URL). In addition, either (i) new pathological Q waves or new LBBB, or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

Source: Thygesen et al (2019)

Physical examination findings

Physical examination can vary from normal to hemodynamic instability including ischemic mitral regurgitation, hypotension, gallop heart sound, jugular venous distension, left ventricular failure, and cardiogenic shock.

Electrocardiograms

Electrocardiogram provides important information about the presence, extent and severity of myocardial ischemia in stratifying the patient risk of ACS and determining the treatment strategy.

Serum cardiac markers

Elevated cardiac biomarkers especially Troponin (I or T), or creatinine kinase (CK) or its isoenzyme MB (CK-MB), reflect myocardial cells necrosis. Troponins have higher clinical sensitivity and specificity than traditional cardiac enzymes. Myoglobin is not specific for the detection of myocardial cell injury (Eggers et al., 2004). Troponin level should be measured within 6 first hours of the onset of pain (strength of recommendation), and the elevation stays for 2 weeks after the onset of myocardial necrosis (Smith et al., 2015). Troponin increase reflects irreversible myocardial cellular necrosis (Eggers et al., 2004). However cardiac biomarkers are not specific of acute MI (Thygesen et al., 2012). Clinical conditions such as pulmonary embolism, heart failure, end stage renal failure, and myocarditis are associated with of cardiac biomarkers increase (Korff, Katus & Giannitsis, 2006).

2.10.2.4. Stable coronary artery disease

Definition, classification

Stable coronary artery disease includes all clinical entities that are characterized by coronary atherosclerosis in the absence of ACS (Abrams, 2005). It is defined as episodes of reversible myocardial demand /supply mismatch, related to ischemia or hypoxia, which are usually inducibleby exercise, emotion or other stress and, reproducible but, may also be occurring

spontaneously (Task members of ESC et al., 2013). The consequences of ischemia are according to a predictable temporal chronology:

• Increased H+ and K+ concentration in the venous blood

• Signs of ventricular diastolic and subsequently systolic dysfunction with regional wall motion abnormalities

• Development of ST-T changes

• Cardiac ischemic pain (angina) (Crea et al., 2010)

In SCAD the symptoms are reversible, repetitive for months to years and relieved by rest or sublingual nitroglycerin. Some conditions such as anemia, hypertension crisis, thyrotoxicosis can exacerbate the angina.

The SCAD definition includes:

• Patients symptomatic for stable angina pectoris or a symptom like angina (e.g. dyspnea)

• Patients with a history of obstructive or non-obstructive CAD, who have become asymptomatic with treatment and need regular follow up

• Patients reporting symptoms for the first time, but already in chronic stable condition (since several months)

Table 2.6: Classification and Severity of Angina

Classification and Severity of Angina according to the Canadian Cardiovascular Society	
Class I (no limitation of ordinary activity)	Angina reproduced with strenuous exertion
Class II (slight limitation of ordinary activity)	Angina reproduced on walking rapidly
Class III (marked limitation of ordinary activity)	Angina reproduced on walking 100-200m
Class IV (inability of activity)	Angina reproduced for any activity

Source: Gerloni et al (2017)

Clinical presentation

The characteristics of angina are:

• Location: retrosternal, or near the sternum, but may be felt anywhere from the epigastrium to the lower jaw or teeth.

• Quality: described as oppressive, a sensation of heaviness, pressure weight, constricting or burning, associated or not by a shortness of breath

• Etiology: exercise or emotional stress, post prandial and cold weather

• Duration: 3 to 15 min (no more than10 min in the majority of cases)

• Remission: by rest or sublingual nitroglycerin

Atypical presentations differ by the absence of precepting factors. The pain starts at rest with a low level of intensity, increases slowly and reaches its peak for a maximum of 15 min. Atypical pain is more common in women, in diabetics and in elderly patients. Women report vague symptoms such as palpitation, inflammatory pain, stabbing pain, which lasts for seconds, hours or days with variable response to nitroglycerin. Diabetic patients are more likely to report dyspnea or to be asymptomatic (Gerloni et al., 2017).

Table 2.7: Traditional clinical classification of chest pain

Typical angina (Definite)	Meets all three of the following characteristics Substernal chest discomfort of characteristic quality and duration Provoked by exertion or emotional stress Relived by rest and /or nitrates within minutes
Atypical angina (probable)	Meets two of these characteristics
Non-anginal chest pain	Lacks or meets only one or none of the characteristics

Source: Diamond (1983)

Investigations

Physical examination is poor and has low sensitivity. A normal ECG does not exclude the diagnosis, but an abnormal resting one increases the diagnosis probability. Routine laboratory tests are recommended to determine the severity of factors. Hyperglycemia, dyslipidemia, thyroid disorder and renal failure should be evaluated in every patient with suspected CAD (Task members of ESC et al., 2013). Plasma cardiac troponin levels are below the normal. According to ESC and Canadian guidelines echocardiography should be performed in all patients with SCAD to identify left ventricular function, kinetic segments, valvular lesion (mainly mitral regurgitation).

The Multidetector row CT permits the detection of coronary calcification. The measurement of calcium scoring is calculated as the coronary calcium area by maximal plaque density (in Hounsfield units) and calcified lesions are quantified using (Agatston score). Calcium scoring helps to evaluate the atherosclerosis burden (Omland et al., 2009; Fihn et al., 2012)

Coronary computed tomography angiography (CTA): is indicated in patients with low to intermediate risk of obstructive CAD. CTA can visualize the coronary arteries after intravenous injection of contrast agent. CTA is helpful in patients without severe obesity, favorable calcium score (Agatston score < 400) and with heart rate $\leq$ 65 beats per minute.

Exercise ECG: with a high specificity > 90% and a low sensitivity < 50% (high probability of false positive mainly in females). The test is usually carried out on a treadmill from rest to maximum exertion according the Bruce protocol. ST segment modification are analyzed (Ashley & Niebauer, 2004).

Stress echocardiography: the predictive value is higher than treadmill test. It detects the difference in wall motion between ischemic and non-ischemic myocardium, and provides information on hibernating myocardium.

Magnetic resonance imaging (MRI): detects wall motion abnormalities

Cardiac Coronary angiography: remains the gold standard. ACS or Positive stress test induced large wall motion abnormality, or poor answer to medical treatment are the main indications. Location and number of lesions, type and degree of stenosis are described (Ashley & Niebauer, 2004).

Strategy diagnostic (Figure 2.12, 2.13)

A Pre-Test Probability (PTP) evaluation prior to non-invasive testing with the Duke clinical score and Diamond Forrest model is recommended in The European and American guidelines (Joseph et al., 2018). PTP of CAD is based upon age, gender and symptoms. It is defined by ESC guidelines as: Low 85%. The intermediate group is classified further into (a) 15-65%, and (b) 66-85% (Montalescot et al., 2013). In the ESC GUIDELINES, CCTA is recommended in patients with a low -intermediate PTP of CAD (15- 50%). Pharmacological stress MRI is a class IIa indication according to the ACC/AHA guidelines*.

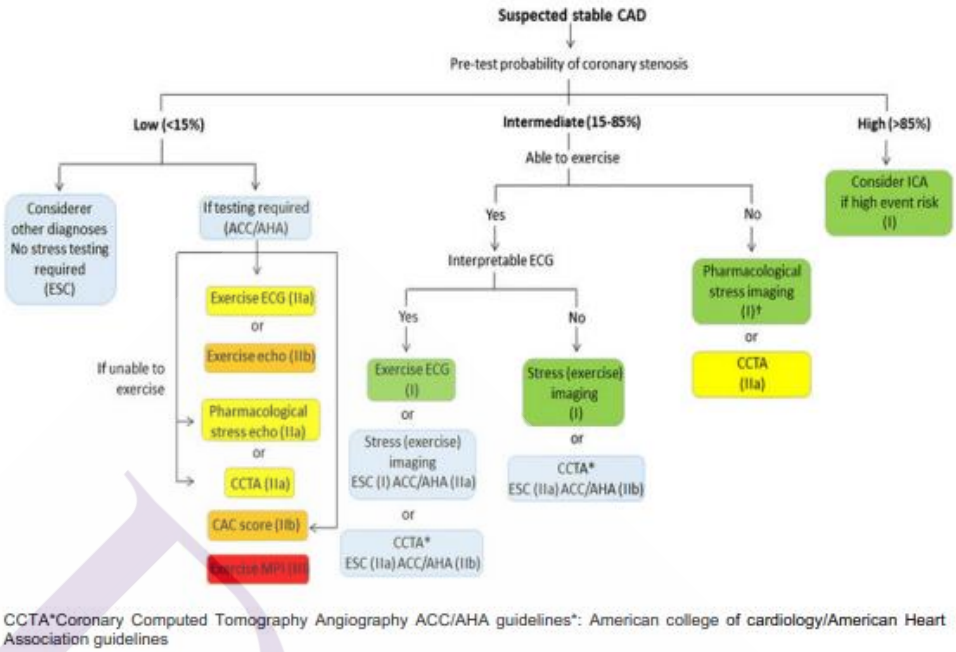


Figure 2.12: ESC and ACC/AHA recommendations for stress testing and CCTA in the assessment of patients with suspected stable CAD according to pre-test probability of disease

Source: Joseph et al. (2018)

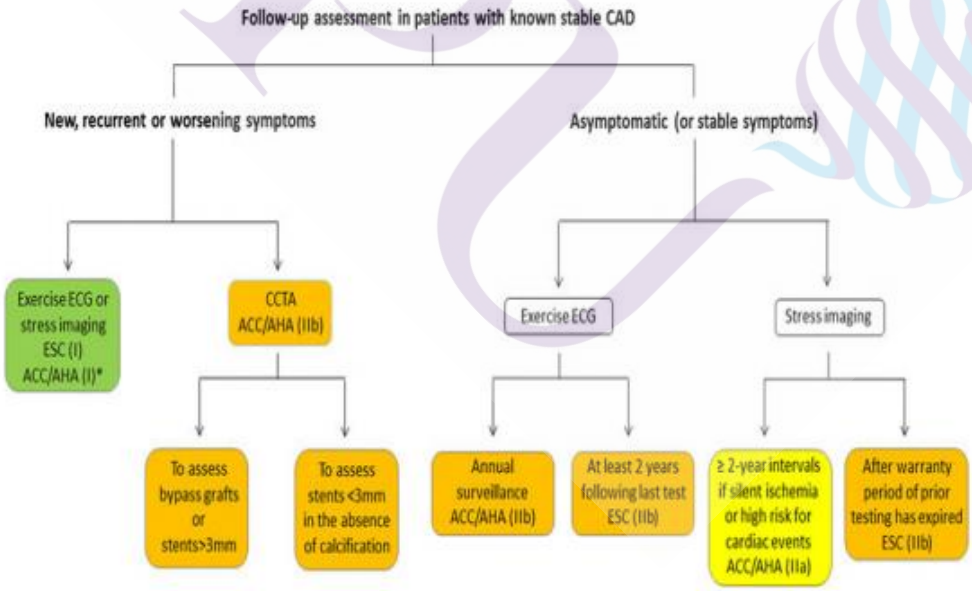


Figure 2.13: ESC and ACC/AHA guidance for follow-up assessment of patients with stable CAD according to symptoms

Source: Joseph et al (2018)

2.11 Risk factors related to Cardiovascular diseases

Definition and classification of cardiovascular risk factors

CVDs are a continuum influenced by cardiovascular risk factors (CVRF) and participate via progressive vascular diseases (CAD, LEAD, stroke) to target organ damage and death. This process leads to two important points: First, the intervention through the circuit can disrupt the pathophysiological process and thus provide cardiovascular protection. Second, the cardiovascular disease in atherosclerosis etiology share the same risk factors, so, it is fundamental to evaluate and treat a patient's total cardiovascular risk rather than considering risk factor independently (Dahlöf, 2010). WHO defines risk factor as a characteristic, condition or behavior that increases the likelihood of getting a disease or injury (WHO Global health risk, 2009). CVRF are defined as characteristics that increase the risk of developing CVD, including two categories modifiable and non-modifiable risk factors

Modifiable risk factors

The world is affected by a global rise in the prevalence of cardiovascular risk factors. Modifiable risk factors are the major contributors to cardiovascular morbidity and mortality including hypertension, smoking, diabetes, obesity, dyslipedemia, stress, unhealthy diet and physical inactivity. These risk factors rarely occur alone, and instead tend to cluster in individuals (Meigs et al., 1997). Recent study reported that only 2%-7% of people are without risk factors, and 70% have multiple risk factors, which increase total individual's risk of CVD; from 4-fold with one risk factors to 60-fold in the cluster of five risk factors (Wilson et al., 1999). The prevalence of multimorbidity (two or more chronic conditions) is increasing, due to growing incidence of chronic conditions and increasing life-expectancy (Uijen & van de Lisdonk, 2008). Number of risk factors were identified in epidemiological surveys. In 2016 the global burden disease for Amal Jamee Shahwan | Ph.D. Thesis | University of Limoges | 2019 License CC BY-NC-ND 4.0 40 risk profiles

in Middle East and North Africa (MENA) stranded out these risk factors by order of priority: high blood pressure ranked as the first, followed by obesity, diabetes then smoking and dyslipidemia (Forouzanfar et al., 2016).

2.11.1 Hypertension

Hypertension constitutes a major public health challenge in the world. HTN is defined as a Systolic Blood Pressure (SBP) $\geq$140 mmHg and /or Diastolic Blood Pressure (DBP) $\geq$ 90 mmHg or taking antihypertensive drugs or having been told at least twice by a healthcare professional as having HTN (Table 5). Normal levels of both systolic and diastolic blood pressure are essential for the function of vital organs such as the heart, brain, kidney, for overall health and wellbeing (WHO, 2013).

Table 2.8: Blood pressure classification according to WHO

Normal	Systolic: less than 120 mmHg Diastolic: less than 80 mmHg
A risk (prehypertension)	Systolic: 120–139 mmHg Diastolic: 80–89 mmHg
High	Systolic: 140 mmHg or higher Diastolic: 90 mmHg or higher

Source: WHO hypertension (2013)

2.11.2 Obesity

Obesity is defined as excess amount of body fat, which leads to an ill health and risk of the development of type 2 diabetes and cardiovascular event. It is related to the epidemiological transition of NCDs. Overall, obesity ranked as the sixth leading cause of Disability Adjusted Life Years (DALYs). Overweight causes almost 3 million deaths worldwide each year (WHO Global health risk, 2009). WHO and National heart, Lung, and blood institute (NHLBI) define weight categories for adults as follows: Body Mass Index (BMI): normal weight (25.0 kg/m2 $\leq$ BMI $\leq$

29.9 kg/m2), obese class I (BMI 30-35 kg/m2), class II (BMI >35 to 39.9 kg/m2), and class III (BMI ≥40 kg/m2) (WHO, 2017a).

2.11.3 Diabetes mellitus

Diabetes mellitus is a condition defined by an elevated level of blood glucose. Glycated haemoglobin A1c (Hb A1c) has been recommended as diagnostic test for DM. The threshold of ≥6.5% was adopted to diagnose diabetes. The classification based on recommendation of the WHO and the American Diabetes Federation (ADF) includes four classes (ESC et al., 2013).

• Type 1 diabetes: results from B-cells destruction leading to insulin deficiency

• Type 2 diabetes: the most common, accounts for 90-95% of all cases. It is the consequence of a progressive insulin secretory defect, in association with obesity, sedentary lifestyle, and insulin resistance

• Gestational diabetes: diagnosed during pregnancy, after delivery, most of them return to a euglycemic state but they are at increased risk for T2DM in the future. A large Canadian study estimates the probability to develop diabetes after 9 months of delivery was 4 % and 19 % after 9 years (Feig et al., 2008)

• Other specific types of diabetes due to other cause such as genetic disorder, diabetes secondary to pathological conditions or disease (pancreatitis, trauma, or surgery of the pancreas), drugs induced diabetes (HIV/AIDS or after organ transplantation)

Table 2.9: Criteria for the diagnosis of diabetes

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h*.
OR
2-h PG ≥200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.‡
OR
A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.‡
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

ıe absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

Source: ADA (2018)

Table 2.10: Type 2 Diabetes Mellitus risk factors

1. Age ≥45years
2. Overweight or obese.
3. Sedentary lifestyle.
4. Family history with diabetes mellitus.
5. History of delivering a baby weighing >9 pounds.
6. Polycystic vary syndrome; and history of diabetes mellitus during pregnancy.
7. Elevated blood pressure (≥140/90 mmHg) or being treated for hypertension.
8. Levels of major lipids: High-density lipoprotein below 35 mg/dL, or TG above 250 mg/dL.
9. Prediabetes HbA1C level of 5.7 to 6.4 %; an elevated FPG test result of 100–125 mg/dL; or a two-hour oral glucose tolerance test result of 140–199 mg/dL.
10. Acanthosis nigricans presenting with a dark, velvety rash around the neck or armpits.
11. Current or prior history of CVDs

Source: ADA (2018)

2.11.4 Alcohol

Epidemiologic studies define “heavy” drinking as ≥ 3 standard drinks per day, and lesser amounts “light” or “moderate” drinking. According to WHO harmful use of alcohol is defined by 6.2 liters of pure alcohol per year which translates to 13.5 grams of pure alcohol per day for persons ≥ 15 years of age (Anon, 2014).

2.11.5 Serum lipids

Dyslipidemia is defined by the elevation or attenuation of serum lipids. Cholesterol and triglycerides are the major lipoproteins. To date, there is no evidence that fasting is superior to non-fasting in evaluating a lipid profile for cardiovascular risk prediction, Many countries are currently in the process of modifying their guidelines for measuring a lipid profile in the nonfasting state, which facilitates blood collection for patients, laboratory technicians and clinician (Langsted & Nordestgaard, 2019; Nordestgaard & Varbo, 2014).

All lipoproteins have a common basic structure but they differs in their size, density composition and chemical proprieties (Yusuf et al., 2004). The different lipoproteins are including chylomicrones, Intermediate Density Lipoprotein (IDL), Very Low-Density Lipoprotein (VLDL), LDL, HDL, and apolipoproteins such as (Apo A, apo B, apo C and apo E). Lipids disorders are defined as the total cholesterol, Low density Lipoprotein Cholesterol (LDL-C), High density Lipoprotein-Cholesterol (HDL-C) and triglycerides. According to the large epidemiological studies, the results of a meta-analysis including 10 large cohort studies reported that for each 0.6 mmol/l or 23 mg/dl reduction in serum cholesterol levels in subjects > 60 years old, decrease the risk of CHD by 27% (Law, Wald & Thompson, 1994).

The National Cholesterol Education Program Adult Treatment Panel III of (NCEP ATPIII) define dyslipidemia as total cholesterol $\geq$ 240 mg/dl, triglycerides > 200 mg/dl, (LDL-C) >160 mg/dl and (HDL -C) < 50 mg/dl in women (N.C.E.P, 2002). The National Health and Nutrition Examination Survey (NHANES) reported a prevalence of total cholesterol level $\geq$ 240mg/dl, in USA population up to 33.6% (Tóth, Potter & Ming, 2012). In Middle east the prevalence of dyslipidemia was 70.5% (Labarthe, 2010; Yusuf et al., 2004). A study among adult population in GCC found that the prevalence of hypercholesteremia defined as total cholesterol >200 mg/dl ranged from 17% to 54.9% in males and 9% to 53.2% in females (Aljefree & Ahmed, 2015). A meta-analysis including 90,056 subjects in 14 randomized trial of statin showed that lowering LDL-C by 39 mg /dl was associated with one-fifth reduction in the 5 years incidence of major cardiovascular events (CAD, and stroke) (Baigent et al., 2005). The negative association between low HDL-C and the risk of heart disease is well defined. In Prospective Cardiovascular Munster (PROCAM) study, subjects with HDL-C < 35 mg/dl have 4-fold higher cardio vascular risk (Assmann et al., 1996). The Israeli Ischemic Heart Disease Study showed that subgroup with low HDL-C concentration had 36% greater CVD mortality than subgroups with elevated HDL-C (even after adjusted for age and CVRF) (Goldbourt, Yaari & Medalie, 1997). In addition a meta-analysis of four studies demonstrated that for every 1mg /dl increase in HDL-C level there was decrease in coronary events risk by 2-3% independently of LDL-C (Gordon et al., 1989).

Triglycerides measurement is important for evaluating the risk of CVD mainly in diabetics, glucose intolerance, and insulin resistance. In the Copenhagen City Heart Study and the Women's Health Study, the increase in non-fasting triglycerides concentration by 5mmol/l versus less than 1mmol/l, was strongly associated with increasing adjusted age risks by 17- fold for MI, by 6 for IHD, 5 for ischemic stroke, and 4 for all-cause mortality in women.

For men the corresponding risks increase were by 5, 3, and 2 fold (Freiberg et al., 2008; Nordestgaard et al., 2007). Non-HDL cholesterol (Non-HDL-C) is the sum of cholesterol collected in all lipoprotein except HDL-C. It is calculated as the difference between total and HDL-C. It should be higher by about 30mg /dl than LDL-C. An elevation of non-HDL-C by 1mg/dl increases the risk of death due to CVD by 5%. It is also considered the second goal after LDL-C in diabetics patients (Bergmann, 2010)

Table2.11: Classification of elevated TG levels

TG category	TG concentration, mg/dL	Goal
Normal	< 150	<150 mg/dL
Borderline-high	150-199	
High	200-499	
Very high	≥ 500	

Source: Jellinger et al (2017)

Table 2.12: LDL-C and non-HDL-C goals in three CHD risk groups

Risk category	LDL-C (mg/dL)	Non-HDL-C (mg/dL)
CHD and CHD risk equivalent (10-years CHD death risk >20%)	**<100**	<130
Multiple (≥ 2) risk factors (10-years CHD death risk <20%)	<130	<160
0-1 risk factor	<160	<190

Source: Graham et al (2007)

2.11.6 Nutrition

Unhealthy diet contains too much fat, sugar, carbohydrates, high fat meats, few Fruits and Vegetables (F&V) and whole grains, without adequate vitamins and minerals. A recent study among 65, 226 English population, found that eating $\geq$ 7 portions of F&V daily reduced the risk of death by heart disease by 31% (Oyebode et al., 2014). The physicians' Health Study, during a follow-up of 12 years, reported 25% lower incidence of CAD in men who consumed >2.5 or more serving of vegetables daily, compared with those who consumed less than one serving daily (Liu et al., 2001). Numerous studies showed that diets high in fiber are significantly associated with lower risks of CVD (stroke and CAD)(Silvia, 2014). Another large prospective cohort study of 84,251 women in the Nurse' Health Study and 42,148 men in the Health Professionals Follow-up Study reported 30% lower risk of CVD in people with highest F&V intake (>5 serving daily) compared to those with lowest intake. For each increase of one serving per /day in F&V, a 4% lower risk of coronary heart disease and 6% lower risk of ischemic stroke (Joshipura et al., 1999, 2001). In addition, decreasing dietary salt intake from 9-12 gram/day to the recommended level of 5 grams/day would have a major impact on BP and CVD (Mendis et al., 2011).

2.11.7 Physical activity

Physically active means that you are protected from numerous risk factors, thus less exposed to CVD. Additionally physical activity is fundamental to energy balance and weight control (WHO, 2010). Morris et al found that civil servant post men who walked or cycled while delivering mail had lower rates of heart disease compared to postal clerks who had sedentarily jobs (Morris & Crawford, 1958). The sedentary life was a predominant factor in industrialized nations (Archer & Blair, 2011). The rise in sedentarism has been an epidemic for chronic disease mainly obesity. Regular physical activity has a protective effect on cardiovascular risk by the deceleration of atherosclerosis progression, improvement of endothelial dysfunction, decreased systemic inflammation and controlling major CVRF such as HTN, diabetes and obesity (Cheng et al., 2013). The prevalence of inactivity was higher among the younger population in GCC ranging from 24.3%

to 93.9% in males and from 50% to 98.1% in females. Saudi Arabia keeps the higher rate (Aljefree & Ahmed, 2015). In a meta-analysis of data from 32 studies comparing inactive persons and those who practiced 150 min /week of moderate - intensity, physical activity had a 14% decrease in CAD, and those who practiced the equivalent of 300 min /week of moderate-intensity physical activity had 20% lower CAD (Sattelmair et al., 2011). In another meta-analysis including 23 studies the risk of stroke (ischemic or hemorrhagic) was 27% in individuals with a moderate activity vs 20% in high physical activity (Lee, Folsom & Blair, 2003).

2.11.8 Stress

Everyone feels stress in different ways and reacts to it in different ways. How much stress you experience and how you react to it can lead to a wide variety of health problems (AHA 2014, 2014). The concept of psychological stress produces a physiological change by the activation of the hypothalamic-pituitary-adrenocortical and sympathetic nervous system, which triggers pathophysiological mechanisms that include inflammation, hemostasis, and dysfunction of metabolic and cardiac autonomic control (Brotman, Golden & Wittstein, 2007). Personality types (type A and type D) lead to unhealthy response to daily stressors. The type D (distressed) personality is a negative affectivity characterized by a combination of pessimistic emotions, depressed mood, anxiety, anger, worried and hostile feelings. This personality is almost related to social phobia and panic disorder (Sher, 2005). By contrast, the type A personality is characterized by anxiety, intense time urgency, intense competitiveness, hypervigilance, and sometimes hostile behaviors (Ragland & Brand, 1988). In the general population, adults with work stress or private-life stress have 1.1-1.6-fold increased risk of CAD and stroke (Kivimäki & Steptoe, 2018). Chronic stress plays a role as a disease trigger in individuals with high atherosclerotic plaque leading to cardiovascular events such as hypertension, insulin resistance, arrhythmia, myocardial ischemia, cardiac failure and stroke Emotional stress is involved in 3.9% of acute cardiac events (Nawrot et al., 2011). The association with stroke was uncertain (Truelsen & Nielsen, 2003). Anxiety was

reported to be high in patients with a history of ACS. The influence lasts for a long period time in 20% to 25% of patients after the first event (Moser et al., 2007).

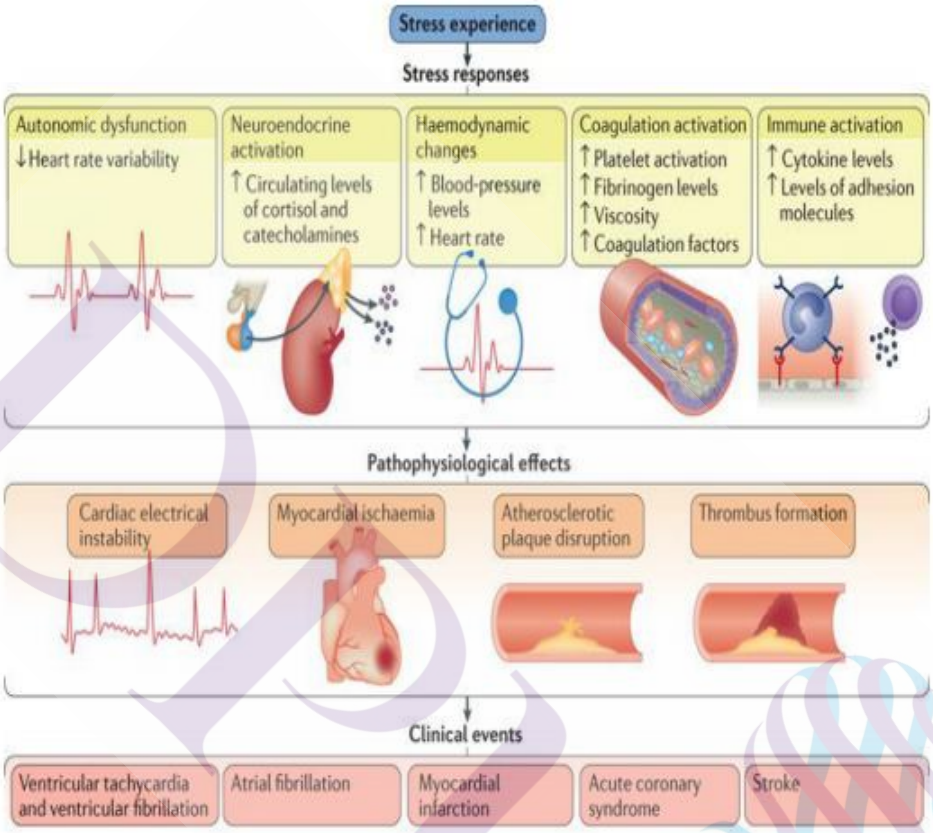


Figure 2.14: Effects of stress on cardiovascular system

Source: Cardiology (2018)

2.11.9 Socioeconomic status

Socioeconomic Status (SES) is a complex concept affecting health and known as powerful predictor of CVDs and death (Stokols, Pelletier & Fielding, 1996). It is measured as a combination of education, income, and occupation but may include age, sex, ethnicity and marital status. The available data showed that the association between SES and CVD depends on the socioeconomic development context, and the stage of the demographic, epidemiological and nutritional transition of the population (Mestral & Stringhini, 2017). In the HIC there is an inverse association between SES and CVD and CVRFs. The SES was measured via education, occupation, or income. However, in LMIC the relation between SES Amal Jamee Shahwan | Ph.D. Thesis | University of Limoges | 2019 License CC BY-NC-ND 4.0 47 and CVD or CDVRFs shows a positive association (Mestral & Stringhini, 2017), because people with lower SES tend to have higher levels of traditional cardiovascular risk factors such as higher BP, smoking, and obesity (Yu et al., 2000). Recent data confirms that SES is closely related with the quality of diet (Turrell et al., 2003). Low SES people prefer white bread, potatoes rice, and refined cereals compared to those with high SES, who prefer whole grain products with lower glycemic index, consumption of vegetables and fruit (Cronin et al., 1982; Shimakawa et al., 1994; Smith & Baghurst, 1992). The behavioral factors, such as physical inactivity, smoking and alcohol intake, explain only 13%-60% of the SES differences in CVD morbidity and 19%-55% of CVD mortality (Méjean et al., 2013).

2.11.10 Non-modifiable risk factors

2.11.10 .1 Age

By 2030 20% of the population will be aged > 65 years. In this age group CVD will result in 40% of all deaths and will be the leading cause (North & Sinclair, 2012). The cardiovascular system is strongly affected by the ageing process leading to progressive deterioration in structure and function of the heart and vasculature that contribute to the development of CVD (Costa et al., 2015). Epidemiologic studies revealed that at any age the risk of cardiovascular events varies

widely (4-5-fold) depending on the associated risk factors. Studies indicate that the chances of surviving to age 85 years have decreased significantly with cumulative risk factors, from 37% for men without risk factors to 2% with five risk factors and from 65% for women without risk factors to 14% with five risk factors (Kannel & Vasan, 2009).

2.11.10.2 Gender

For many years, CVD was considered as a male disease. However, in the European population 38% of cardiovascular deaths before the age of 75 years were in women and 37% in men (European Cardiovascular Disease Statistics, 2012). The difference between male and female was previously described in epidemiology, pathophysiology, clinical manifestation and management of CVD (Anon, 2012c). The sexual hormones drive differences in gene expression and the function of cardiovascular system (Regitz-Zagrosek et al., 2016). In male sex, cardiovascular risk increases over time, as well as atherosclerosis process continues. In contrast women are protected from atherosclerosis during the fertile age by estrogens that exert favorable effect on cardiovascular system. The effect disappears after menopause. Women and men manifest a similar cardiovascular profile with a difference of 10 years of age (Perk et al., 2012). In 2004, the WHO reported a total cardiovascular mortality of 55% in women and 43% in men. IHD, stroke and other CVD represent 23%, 18% and 15% respectively in women and 21%,11% and 11% respectively in men (Stramba-Badiale et al., 2006).

Over 5 decades in the Framingham study cohort the occurrence of CAD or stroke were up to 47%-31% and 15%-18% respectively in males and females. The higher risk of stroke in female was related to longer life expectancy (Lloyd-Jones et al., 1999; Seshadri et al., 2006). Gender difference for traditional cardiovascular risk factors associated with CVD were also documented. Age, hypertension, total cholesterol and LDL-C had a great influence in men, while menopause, systolic arterial hypertension, smoking, diabetes, triglycerides and HDL-C were the main actors in women (Leonarda & Gabriella, 2015). Every 10 mmHg of SBP was associated with15% increased risk of CAD and 25% increased risk of stroke in both sexes (Peters, Huxley & Woodward, 2013).

Diabetes increased cardiovascular risk of 3-7 folds in women and 2-3 folds in men (Manson, 1996). Two large meta-analyses reported that the risk of CAD and stroke were increased by 44% and 27% in diabetic women (Huxley, Barzi & Woodward, 2006; Peters, Huxley & Woodward, 2014). The prevalence of overweight in men and women differs according to the level of the development of the country. Higher BMI is more prevalent in men than women in HIC, conversely in LMIC mainly in Arab countries a female predominance was described (Anon, 2015). Total cholesterol confers the same risk of cardiovascular in both sexes.

However, LDL-C increases cardiovascular risk in men more than women (Manolio et al., 1992). Low HDL-C represents equal risk for CAD in both sexes, mainly young age, but predicts CAD mortality in women more than in men. In addition, triglycerides are a part of metabolic syndrome which is higher in women. Smoking increases risk of cardiovascular events by 3.6 in women and 2.4 in men (Willett et al., 1987). A meta-analysis from 74 prospective cohort studies show that women who smoke had a 25% greater relative risk of CAD than men (Huxley & Woodward, 2011)

2.11.10.3. Family history

Represents one of the main risk factors for CVD, especially in the younger population with a first-degree relative disorder: (men below the age of 55 years and women below 65 years) (Choudhury & Marsh, 1999; Elis & Lishner, 2004). Family history helps to define the small subset of families that account for the majority of prevalent cases in the population (Hunt, Gwinn & Adams, 2003). The risk for CVD and stroke among subjects with positive family history ranges from 2 to 9 and 1.5 to 2, respectively (Kardia, Modell & Peyser, 2003; Liao et al., 1997). Family history represents the interaction between genetic, environmental and behavioral factors (Elis et al., 2008). Even a non-premature parental history increases the risk of CVD in offspring (Sesso et al., 2001).The history of heart attack in both parents increases the risk of CAD mainly when 1 parent has a premature coronary event before 50 years of age (Chow et al., 2011). Sibling history of CVD has been shown to increase the odds of CVD in males and females by 45% (Murabito et al., 2005).

In a recent study of patients with premature ACS (age $\leq$ 55 years), 28% of the females and 20% of the males had a family history of CAD. Patients with family history of CAD had a higher prevalence of traditional CVD risk factors (HTN, DM, dyslipidemia and obesity) (Choi et al., 2014; Hunt, Gwinn & Adams, 2003; Yoon et al., 2002). In monozygotic twins the risk of death from CAD increased 3.8 to 15 times if a sibling died of CAD before age 75 (Marenberg et al., 1994). In addition a large international case-control study reported a rise in the risk of MI if one parent had MI (OR=1.67), or one parent had MI before age 50 (OR=2.36), or both parents had MI (OR=2.90) and if both parent had MI before age 50 (OR=6.56) (Anderson et al., 2013). The prevalence of a positive family history ranges from 14% to 35% in the general population, 75% of those with premature heart disease have a positive family history highlighting the opportunities for prevention (Hawe et al., 2003).

2.11.10.4 Menopause

Menopause is an indicator of the transition from reproductive to non-reproductive life and is associated with biological and hormonal changes. The risk is related to post menopause is due to a sudden decrease of estrogen hormone, which has protective effects on lipid, glycemic metabolism and vessels (Rossi et al., 2002). Menopausal status and estrogen deficiency were frequently associated with hypertension due to increase in BMI, insulin-resistance, sodium retention, and with increased smooth muscle cell proliferation leading to an increase in systemic vascular resistance. The role of Hormonal Replacement Therapy (HRT) in CVD is controversial. In contrast Women's Health Initiative study described an increased risk of CAD and breast cancer in users of HRT including estrogens and a synthetic progestin (Rossouw et al., 2007; The Writing Group on behalf of the Workshop Consensus Group, 2009)

2.11.11 Novel risk factors

2.11.11.1 Fibrinogen

Plasma fibrinogen as a coagulation factor is a heterogeneous mixture of many different molecular forms (Gordon et al., 1989). Many studies reported the strong relationship between plasma fibrinogen concentration and cardiovascular disease mainly CAD, stroke and LEAD (Lowe, 1995). Fibrinogen concentration raises the risk of atherogenesis, thrombogenesis and ischemia (Danesh et al., 2000). The fibrinogen is a pathway by which traditional risk factors exert their effect. For example, fibrinogen levels increased risk of CVD associated with smoking and essentially with the number of cigarettes smoked. The level decreases after smoking cessation (Fogari et al., 1994). The risk of CVD associated with obesity might be driven by the fibrinogen. A loss of weight after low calories diet leads to fall in fibrinogen levels (Ditschuneit, Flechtner-Mors & Adler, 1995).

Prospective Cardiovascular Munster (PROCAM) study found that subjects with both high level of LDL-C and fibrinogen had a 6.1-fold increase in CAD compared with those with lower or normal levels (Heinrich et al., 1994; Thompson et al., 1995). The Gothenburg and Framingham Studies reported that plasma fibrinogen levels represent an independent risk factor for MI and stroke and strong risk factor for cardiac sudden death in patients with CAD (Thompson et al., 1995). Also, platelets hyperactivity due to high fibrinogen concentration was found in diabetic patients (Stec et al., 2000).

2.11.11.2 Homocysteine

Homocysteine concentration is higher in men than women and increases with age. The difference becomes apparent in puberty and it is related to hormonal factors, lifestyle, nutrition, and vitamins. Thromboembolic events were present in 50% of untreated persons with high level of homocysteine, and 20% die before the age of 30 years (Nygård et al., 1999). Numerous clinical studies demonstrated a relationship between total homocysteine levels and CAD, LEAD, stroke or venous thrombosis (Boushey et al., 1995; Verhoef & Stampfer, 1995). The homocysteine affects the coagulation system and the resistance of the endothelium to thrombosis and may interfere with the vasodilator and antithrombotic effects of nitric oxide (Stamler & Slivka, 1996).

2.11.11.3 C-Reactive Protein

C-Reactive Protein (CRP) is a marker of inflammation and a hepatically derived pentraxin that plays a role in the immune response. CRP has a long plasma half-life. Numerous epidemiological studies have demonstrated the role of CRP in the occurrence of MI, stroke, LEAD, sudden cardiac death and it plays a role in almost all process associated with metabolic syndrome (Ridker, 2003). CRP seems to be a stronger predictor of cardiovascular events than LDL ch (Mendall et al., 2000). CRP levels 3 mg/dl correspond to low-moderate, and high risk. Subjects with LDL ch 3 mg /dl represent a high-risk group (Ridker, 2003). CRP was a strong predictor of risk even 20 years after initial blood samples were obtained (Sakkinen et al., 2002).

2.11.12 Co-occurrence of risk factors

A number of studies have demonstrated that five modifiable CVRF such as cigarette smoking, overweight or obesity, hypertension, diabetes and dyslipidemia can be eliminated by management. The risk of CVD increases with increasing number of risk factors (Yusuf et al., 1998). Data from the first National Health and Nutrition Examination Surveys Epidemiologic Follow-up study showed that the risk for CVD increased with each additional risk factor. More than 50% of the incidence of CAD, stroke and all-cause mortality was due to having one risk factor and the risk increases up to 70% for individuals with three risk factors (Yusuf et al., 1998). Primary prevention and control risk factors may not reduce the risk of CVD to the equivalent of never having a risk factor. Individuals who control their hypertension are at high risk for CVD compared with those who never develop HTN (N.H.BP.E.P, 1993). Furthermore, the Metabolic Syndrome (MetS) was identified by several criteria and defined as an asymptomatic, pathophysiological state of chronic inflammation, and a cluster of the most harmful risk factors, such as obesity, insulin resistance, hypertension, hyperglycemia and dyslipidemia (Kaur, 2014). The International Diabetes Federation (IDF) estimates that a quarter of the world's adult population has MetS (O'Neill & O'Driscoll, 2015). The rate varies depending on age, ethnicity, and gender of the population (Kaur, 2014). MetS confers a 5-fold increase in the risk of type 2 diabetes mellitus and 2-fold the risk of developing

CVD over the next 5 to 10 years (Alberti et al., 2009). In addition individuals with MetS are at 2-4 fold the risk of stroke and 3-4 fold the risk of myocardial infraction (Alberti, Zimmet & Shaw, 2005). The early identification and control of MetS components prevents the development of the syndrome and reduces CVD events.

2.12 Epidemiology of Cardiovascular disease and cardiovascular risk factors

Cardiovascular disease (CVD) is a major public health burden and is the leading cause of total deaths in all regions of the world except Sub Saharan Africa. In 2016 the number of people dying from CVD increased by 15% accounting for 17.6 million deaths per years, due to population ageing and growth (GBD 2016 Causes of Death Collaborators, 2017), and projected to increase to 23.6 million by 2030 (WHO 2014, n.d.) with total cost of $863 billion (Bloom et al., 2012). Ischemic heart disease and stroke combined account for more than 85.1% of all CVD death in 2016 (GBD 2016 Causes of Death Collaborators, 2017). Coronary artery disease was the most common 45.1%, followed by stroke 16.5% (CDC, 2016a). In USA adults' population 92.1 million have at least one type of CVD (Benjamin et al., 2017). By 2030, 43.9% of USA population is projected to have some form of CVD (Heidenreich et al., 2011). The prevalence of CVD in USA population >25 of age in 2011-2014 was 36.6% (Benjamin Emelia J. et al., 2018). In Europe CVD account for 45% of all deaths in Europe and 37% of all death in the European Union mainly (Wilkins et al., 2017). In United kingdom in 2012, CVD represented a second cause of deaths (28%), 46% were from CAD and 26% were from stroke (Bhatnagar et al., 2016). Major CVD event rates, CVD death rates, and all cause death rate were lowest in HIC and highest in LMIC accounting for 70% of CVD death (WHO 2014, n.d.).

CAD remains the main cause of death up to 13.2% of total deaths worldwide (Usta & Bedel, 2017). In the United State of America (USA), it accounts for one quarter of all deaths. In the European countries 27%-34% of people with CAD are over 75 years (Members et al., 2002). In United kingdom (UK), CAD was responsible for 16% of all male deaths and 10% of all female deaths (Bhatnagar et al., 2015). The mortality rate has declined in HIC, in the SWEDEHEART

registry the mortality related to ACS has decreased over the last three decades (Szummer et al., 2017). Total CAD prevalence was 6.3% in USA adults ≥ 20 years of age (7.4% in males and 5.3% in females) (Benjamin Emelia J. et al., 2018). There is a strong relation between sudden death and coronary disease. Post mortem studies and death certificates revealed that 62-85% of patients who died out of hospital have past history of CAD (Sanchis-Gomar et al., 2016). According to data from NHANES 2011 to 2014, myocardial infraction has a prevalence of 3.0% (3.3% for males and 2.3% in females) in adult USA population (Benjamin et al., 2017). Approximatively every 40 seconds, an American will have an MI. The average age at first MI in American population is 65.6 years for males and 72.0 years for females (Benjamin Emelia J. et al., 2018)). In Middle East the rate of death from CAD was higher compared to western countries United Kingdom (UK), Germany, and the USA. The INTERHEART study (an international case-control analysis of the risk factors for the first MI carried out in 52 countries) found that the median age at MI onset was 51 years in the Middle East population, and was 12 years lower than the median age at presentation in western countries (Gehani et al., 2014).

Cardiovascular risk factors in the world

Hypertension

Approximately one billion of people worldwide have HTN which corresponds to more than 40% (Mozaffarian et al., 2016) and projected to increase by 30% in 2025 (Kearney et al., 2005). In all world regions the prevalence is similar in males and females and rises with increasing age and BMI. Globally HTN is the major cause of mortality responsible for over 7.5 million deaths annually (WHO Global health risk, 2009). The NHANES 2011-2014 estimates the prevalence of USA adults aged ≥ 20 years was up to 34%, ranging from 11.6% in those aged 20-39 years to 67.2% among those aged ≥ 60 years (Benjamin Emelia J. et al., 2018). In LMIC 1 in 3 persons have HTN, with higher rate in elderly and overweight or obese person (Sarki et al., 2015). In Middle East the prevalence of HTN is 30% with slight difference between sexes (30.7% in male and 29.1% in female) (Anon, 2012a). In North Africa and Middle East, HTN was among the three leading risk

factors for disease burden in 2015 as well as in Palestine (Forouzanfar et al., 2016). In the Africa Middle East Cardiovascular Epidemiology study (ACE), conducted in 14 countries of Middle east and Africa (2012), the prevalence of HTN ranged from 25% in Tunisia to 53% in South Africa (Alsheikh-Ali et al., 2014).

Hypertension and cardiovascular disease

Several studies have reported the association between either SBP or DBP and the increase CVD risk. There is no threshold at which the risk becomes apparent. Stroke, CAD, left ventricular hypertrophy, LEAD and chronic kidney disease are the main complications of HTN. An increase of 20 mmHg in SBP or a 10 mmHg increase in DBP was associated with a 2-fold increased risk of death from stroke, heart disease or other vascular disease (Rapsomaniki et al., 2014). Individuals with high-normal BP (130-139/85-89mm Hg) have a 3-fold greater risk of progression to HTN and 2-fold increase risk of CVD (Julius et al., 2006).The risk of CVD associated with hypertension is observed from 30 years to 80 years of age (Whelton et al., 2017). In the Framingham Heart Study during 36-years of follow up HTN was associated with a 2 to 4 fold increase of cardiovascular events in men and women equally (Kannel & Wilson PWf, 2003). HTN is closely associated with the risk of stroke, and it is the commonest factor for end stage renal disease. Controlling BP alone decreases the risk of stroke by 30% and MI by 20%-25% (Tailakh et al., 2014).

Obesity

In 2016, 39% of adults aged 18 years (39% of men and 40% of women) were overweight and 13% of the world's adult population (11% of men and 15% of women) were obese (WHO, 2017a). The prevalence of obesity in 2015-2016 among American adults was 39.6% and 4 in 10 adults were obese (Hales et al., 2017). The Middle East region is affected by alarming increase in the prevalence of obesity at all ages, mainly in the Arab countries (Ali et al., 2013; Ng et al., 2011), where the prevalence is close to that found in western countries. The areas with the higher rate were

Jordan (49.7%), Palestine (41.5%), Qatar (40.8%),Tunis (34%) and Oman (30.8%) (Elasmi et al., 2009; Motlagh, O'Donnell & Yusuf, 2009).

Obesity and cardiovascular disease

Overweight or abdominal obesity causes or exacerbates other cardiovascular metabolic risk factors including hypertension, diabetes, dyslipidemia. These risk factors in turn, increase the likelihood of morbidity and mortality from CVD and contribute to increased health care costs (Cannon, 2008; Tangalos, Cota & Fujioka, 2006). Adiposity is the result of the balance between energy intake and energy expenditure. The rapid rise in the rate of obesity is driven by increased total energy intake, sedentary life or both (Canoy & Buchan, n.d.). Higher body mass index (BMI) was associated with premature mortality. Non-smokers who were obese at age 40 years died 6-7 years earlier than no obese (Peeters et al., 2003). Another important factor associated with obesity is the socio-economic status (SES). In developing countries obesity is more prevalent in women of higher SES, but the epidemic affects lower SES when high fat diet becomes more affordable (Prentice, 2006). Higher levels of education are associated with lower rates of obesity in HIC (Mitchell & Shaw, 2015). Physical inactivity and sedentarily lifestyle are added to the above risk for the development of obesity (Yusuf et al., 2004). Obesity is associated with increase prevalence of type 2 DM, HTN, dyslipidemia, sleep-disorder breathing, CAD, stroke, atrial fibrillation and dementia (Benjamin et al., 2017). In USA population data from NHANES showed that DM type 2 was 18.5% in obese adults, 8.2% in those who were overweight, and 5.4% in normal weight. The prevalence of HTN were 35.7%, 26.4% and 19.8% respectively. Prevalence of dyslipidemia was 49.7% in obese adults, 44.2% in overweight, and 28.6% in normal weight (Saydah et al., 2014). Cardiovascular risks was higher with class III obesity than with class I obesity (McTigue et al., 2014) .

Diabetes

According to The WHO, diabetes is the leading cause of death in the world and the similar trend was available from the Arab world (Abuyassin & Laher, 2016). Data from (IDF) estimated that 415

million adults aged 20-79 years have DM in 2015 and the number will reach 642 million in 2040 with a rise in prevalence from 8.8% to 10.4. This prevalence accounts for 3.3% in Africa,7.3% in Europe, and 10.7% in Middle East and North Africa (Fan, 2017). In USA 9.4% of adult population had diabetes and 25.2% among those $\geq$ 65 years of age (CDC, 2018). WHO estimates that 58% of diabetes mellitus occurs in individual with BMI >21kg/m2 (Mokdad et al., 2000). The prevalence of diabetes in the GCC ranged from 6% to 23.7% (Aljefree & Ahmed, 2015). In the MENA region (2015), four out of ten adults with diabetes are undiagnosed, and approximatively 9.3% (6.3-12.2%) of adults aged 20-79 years are living with diabetes, over 40.6% of them are undiagnosed (Majeed et al., 2014). For Arab countries, the prevalence of type 2 diabetes was 25.4%,17.9%,19.7% and 15.1% in Arabia Saudi, Kuwait, Iraq and Tunis respectively (Alarouj et al., 2013; Al-Rubeaan et al., 2015; Ben Romdhane et al., 2014; Mansour et al., 2014). The countries with the largest number of adults with diabetes are Egypt, Pakistan and Iran (7.8,7.0 and 4.6 million)(IDF 2015, 2015). In 2015 a Tunisian study revealed that the prevalence of type 2 DM will reach 26.6% in 2027 (Saidi et al., 2015). And the Saudi study indicates that the prevalence of type 2 DM in Saudi Arabia will increase to 44.1% in 2022 (Al-Quwaidhi et al., 2014). This means that serious and effective action on obesity and other risk factors must be taken. The IDF evaluates a total health care cost for diabetes in many countries for 5-10% of the total budget (Alberti, Zimmet & Shaw, 2007).

Diabetes and cardiovascular disease

CVD is the most prevalent cause of mortality and morbidity in diabetic populations (Matheus et al., 2013). The relative risk in adults with diabetes ranges from 1 to 3 in men and from 2 to 5 in women compared to those without diabetes (Rivellese, Riccardi & Vaccaro, 2010). Diabetic subjects have twice to four times risk of death from heart disease, 68% in age > 65 years die from CAD and 16% from stroke (AHA 2018, n.d.). The death accounts for 44% in type 1 DM and 52% in type 2 DM (Morrish et al., 2001). Actually AHA/ACC and ESC consider diabetes as a CAD risk equivalent (Piepoli et al., 2016; Stone et al., 2013). Cardiovascular risk factors such as obesity, hypertension and dyslipidemia are common in diabetic patients (Leon & Maddox, 2015).

Several studies reported that increased factor like oxidative stress, coagulability, endothelial dysfunction and autonomic neuropathy are associated with DM and lead to the development of CVD (Matheus et al., 2013). Individuals with diabetes and with poor control suffer from microvascular and macrovascular complications (Matheus et al., 2013)

Smoking

The most common form of tobacco use are cigarette smoking, electronic cigarette (e-cigarette) involving the inhalation of a vaporized liquid that includes nicotine, solvents, and flavoring cigarillos, water pipe and hookahs (CDC, 2016b). This addictive practice is a well-known cause of cancers, cardiovascular, and respiratory diseases (Office of the Surgeon General (US) & Office on Smoking and Health (US), 2004). Cigarette smoking increases inflammation and thrombosis leading to oxidative stress manifestation, prothrombotic activity, platelet aggregation, leukocyte activation, lipids peroxidative and smooth muscle proliferation (Ambrose & Barua, 2004). Nicotine affects the cardiovascular system by increasing systolic and diastolic blood pressure, heart rate and cardiac output (Filion & Luepker, 2013). The WHO estimates the mortality rate associated with tobacco smoking to be seven million people per annum projected to increase by eight million in 2030, while around 890,000 are the result of on-smokers being exposed to second-hand smoke. Nowadays around 80% of smokers live in LMIC (WHO,Tobacco 2018, n.d.). The rate is 5 times higher in men than in women (48% vs.10%) (Hitchman & Fong, 2011). In the EMR, the prevalence of smoking reported in 21 studies was 15.6% and still more common in men than women (28.8% vs.2.9% respectively) (Motlagh, O'Donnell & Yusuf, 2009). Jordan and Tunisia have the highest age standardized tobacco use (36% and 26% respectively), where Oman has the lowest (11%) (Mandil, Chaaya & Saab, 2013). In many Arab countries half of the male population smokes cigarette with a gender based ratio of 10:1 (Eriksen, Mackay & Ross, 2013). This inequality is due to the unacceptability of smoking among females in the culture of Arab countries (Tamim et al., 2007). In contrast women and youth group use water pipe smoker (Maziak et al., 2014). In a longitudinal study smoking behavior among youth in the region, water pipe

smoking prevalence was more than double that of cigarette at baseline (13.3% vs.5.3%) and increased by 40% within 2 years of follow-up from (13.3% to 18.9%) (Mzayek et al., 2012). In the Gulf Cooperation Council the rate of smoking ranged from 13.4% to 37.4% in males and from 0.5% to 20.7% in females (Aljefree & Ahmed, 2015).

Smoking and cardiovascular disease

Cigarette smoking is the major cause of CVD. It influences other cardiovascular risk factors and predisposes individuals to different clinical atherosclerosis syndrome such as CAD, stroke and LEAD, but the relative risk for each disease varies with the vascular bed. The risk is greatest for LEAD, lower for stroke and intermediate for CAD (Health, 2014). The European data indicates that smoking doubles the 10 years CVD mortality rate. The presence of smoking alone doubles the level of risk. The addition of other major risk factors with smoking results in approximately a 4-fold (2x2) increase in risk and the presence of 2 other risk factors together with smoking leads to an 8-fold (2x2x2) increase risk (Burns, 2003). Cigarette smoking along with diabetes are well known as the major risk factors for symptomatic and asymptomatic LEAD. In addition cigarette smoking has been associated with progression of LEAD over a 4- years interval (Hooi et al., 1998; Palumbo et al., 1991)

Table 2.13: Tobacco or secondhand smoke and CVD risk

- For every cigarette smoked, the risk of a non-fatal heart attack increases by 5.6%.
- The risk of heart attack is likely to be more than double by chewing tobacco.
- Breathing secondhand smoke increases in non-smokers the risk of developing a CVD by 25–30%.
- Secondhand smoke contributes to 600,000 deaths annually, of which 28% are children
- The risk of a heart attack is almost doubled by frequent expo-sure to tobacco smoke at workplace or home.

Source: World Heart Federation (2017)

Alcohol

In 2012, 5.9% of all global deaths were attributable to alcohol intake with significant sex differences (7.6% and 4%) among males and females, respectively (Anon, 2014). Alcohol dependence is a major health and social issue in the European union which is the heaviest drinking region in the world (Anon, 2014). Excessive drinking is the third leading cause of premature death after smoking and obesity and approximately 30% of the US population are excessive drinkers (Stahre et al., 2014). Heavy drinking doubles mortality rate, and consumption of 3-5 drinks is associated with a 50% higher mortality rate compared with nondrinkers (Mukamal et al., 2010). Alcohol consumption is associated with an increase in HDLC, a decrease in Low-density lipoprotein Cholesterol (LDL-C), and fibrinogen levels, which thus reduces platelet aggregability (Brien et al., 2011; Movva & Figueredo, 2014). Arab countries have a strict regulation on the sale and consumption of alcohol.

Alcohol and cardiovascular disease

Confusion and controversy are seen in numerous studies about the role played by alcohol consumption in the etiology and prognosis of cardiovascular events (Klatsky, 2015). In the INTERSTROKE study, including 13,447 stroke cases and 13,472 controls from 32 countries, the risk for stroke in low-moderate alcohol use ($\leq$14 drinks per week in women and $\leq$ 21 drinks per week in men) were (1.14 and 1.43) in men and women respectively. In high alcohol intake (>14 drinks per week in women and >21 drinks per week in men) the risks were 2.09 and 2.44 respectively (O'Donnell et al., 2016). However in Prospective Urban Rural Epidemiology (PURE) study which involved 114,970 adults from high, middle, and low income countries showed a neutral relationship between alcohol use and stroke risk (Smyth et al., 2015). Taken together these two large epidemiologic studies indicate that even small amount of alcohol do not protect against stroke, or cardiac event (Toma, Paré & Leong, 2017). Also, in the Health Professionals Follow up Study during 16 years, including 8867 physicians men free of major illness, moderate alcohol intake was associated with lower risk of MI (Mukamal, Chiuve & Rimm, 2006). Keeping alcohol consumption

less than 46 g /day in men and 23 g /day in women appears to minimize the risk of mortality in Japanese population (Inoue et al., 2012). In Second Manifestation of ARTerial (SMART) study, moderate alcohol intake (1-2drinks/day) was associated with decrease risk of vascular, non-fatal events from CAD, stroke, amputation and all-causes of death (Beulens et al., 2010).

2.13 The Framingham Heart Study (FHS) and Framingham Risk Score (FRS)

The Framingham Heart Study (FHS) began in 1948. It was named for Framingham, a town in eastern Massachusetts that was selected as the site of the study. The project was initiated under the direction of the National Heart, lung and Blood Institute and with the collaboration with the Boston University School of Medicine. The goal of the study was to identify common risk factors or characteristics that contribute to CVD. The study included two-third of the adult population (more than 5,200 residents of Framingham city) with ages ranging from 30-62 years. In 1972 more than 5,120 individuals (offspring) of original study participants and their spouses were added in the cohort. In 2001 a third-generation cohort, consisting of grandchildren of the original cohort was added to explore genetic factors to deepen the research in CVD. Additionally, the study recruited the OMNI1 and OMNI2 cohort in1994 and 2003, respectively aimed to reflect the racial and ethnic diversity of the town of Framingham. Every two years persons enrolled in the study were submitted to medical exams and detailed questionnaire about their lifestyle (Tsao & Vasan, 2015). In 1961, Dr William Kannel, director of FHS published the first report on coronary heart disease risk associated with age, male gender, HTN, high cholesterol, DM and electrocardiographic left ventricular hypertrophy (Kannel et al., 1961). Also, reports on HTN and its relation to CVD, studies on lipids and relation to CAD, and life style factors and their implications for CVD were published. All this collective data constitute a foundational knowledge base to guide public health efforts on CVD prevention (Doyle et al., 1962; Hubert et al., 1983; Kannel & Sorlie, 1979; Pencina et al., 2009). Also, from FHS numerous scores were established.

2.13.1 The Prospective Urban Rural Epidemiology (PURE) study

The PURE study is a large epidemiological study that involved 150,000 adults aged 35-70 years residing in approximately 600 communities selected from 17 countries: 3 HIC, 7 MIC and 7 LIC, around the world. Within this context, the PURE study was designed to collect data on socioeconomical status, medical history, lifestyle behaviors (smoking, physical activity, diet) anthropometrics measure, biological and genetic factors (Teo et al., 2009). Also, PURE looks at countries grouped by socioeconomic status and the differences between rural and urban communities (Spencer, 2014). The main results revealed:

• That major CVD, fatal CVD and death from any cause are higher in LIC than in HIC

• PURE confirm that the burden of total CVD is similar in HIC, MIC and LIC

• Regarding the difference between rural and urban areas, PURE found that the burden of CVD was lower in urban communities, but the rate of non-major cardiovascular events was higher in urban areas. This is probably due to the high availability of hospitals in urban areas and better access to health care which explains the lower cardiovascular mortality rate than in rural areas

• Better control of HTN in HIC compared with LIC (Chow et al., 2013)

• PURE indicates that secondary preventive drugs (antiplatelets drugs, B blockers, ACEIs, ARBs and statin) even at low-cost are less likely to be used in LIC than in HIC (Yusuf et al., 2011)

2.13.2 Cardiovascular risk scoring models

The cardiovascular risk scoring gives an estimate "of the probability that a person will develop cardiovascular disease within a specified amount of time" (Wikipedia, 2018). Because these scores give an estimation of the risk of developing CVD, they also indicate who is most likely to benefit from prevention.

2.13.3 Framingham Risk Score

The Framingham Risk Score (FRS) is an older popular tool widely used in clinical practice and research studies. It calculates the risk of CAD events (angina, myocardial infarction, and coronary death) over a 10 years period in asymptomatic patients. Risk factors used in Framingham scoring include age, sex, total cholesterol, HDL cholesterol, smoking, and blood pressure. The score has been validated in many populations, such as Caucasian Americans and African- Americans. This score was implemented in several guidelines for CVD prevention and has been used to guide treatment of risk factors (Berger et al., 2010; D'Agostino et al., 2001).

2.13.4 Global Cardiovascular Framingham Risk

Recently, the Framingham investigators presented a modified score for the estimation of the global CVD including (CVD death, general CAD, ischemic stroke, hemorrhagic stroke, transient ischemic attack, peripheral artery disease and congestive heart failure), based on age, diabetes, smoking, SBP, treated and untreated BP, total cholesterol and HDL cholesterol; it applied to both sexes without previous history of CVD (D'Agostino et al., 2008). A10-year risk score can be derived as percentage which can be used to inform the decision initiating lipids lowering drugs for primary prevention. The risk is considered low if the score is <10%, moderate (10%-20%) and high ≥20% (D'Agostino et al.,2013)

2.31.5 Systemic Coronary Risk Evaluation (SCORE)

SCORE was derived from 12 different European cohort studies, with a large number of participants (250,000). The SCORE system predicts the10-year risk of a first fatal CVD event including heart attack, stroke or aortic aneurysm. Risk factors used in this score system include age, gender, total cholesterol to HDL cholesterol ratio, SBP, and smoking (Piepoli et al., 2016). Two charts are proposed considering the overall characteristics of the population (high or low risk).

2.13.6 WHO / ISH cardiovascular risk prediction charts

The WHO/ISH risk prediction charts indicate 10-year risk of a fatal or nonfatal major cardiovascular event (Myocardial infraction or stroke), help to identify those at high cardiovascular risk. High risk score motivates patients to change behavior and to take antihypertensive drugs, lipid lowering drugs and aspirin in people without CAD, stroke or other atherosclerotic disease. The score is based on age, sex, blood pressure, smoking, total cholesterol and the presence or absence of diabetes mellitus for 14 WHO regions, mainly in LMIC populations. There are two types of charts: one with lab test where the cholesterol can be measured, the other one without lab test in which blood cholesterol cannot be measured (Anon, 2007). The color of the cell indicates the 10-year risk as shown: Green (< 20%), Orange (20% to < 30%), Red (30% to < 40%), Deep Red ($\geq$ 40%).

2.13.7 Atherosclerotic Cardiovascular Disease risk calculator (ASCVD Risk) ACC/AHA)

The ASCVD is defined as nonfatal myocardial infarction, coronary heart disease, or stroke. The Pooled Cohort Equation (PCE) estimates the 10-year primary risk of ASCVD event developed by the American College of Cardiology /American Health Association. It was validated among Caucasian and African American and used only for adult patients without clinical ASCVD in age between 40-79 years (Goff et al., 2014). The risk factors used in this model were age, gender, race, total cholesterol, HDL cholesterol, SBP, treatment of BP, diabetes mellitus and smoking. Patients are considered to be at elevated risk if the pooled cohort equation predicts a risk of >7.5%, in this condition the 2013 ACC/AHA guidelines recommended the use of statin (Stone et al., 2013).

2.13.8 Coronary Heart Disease risk equivalents

Defined as patients with a 10 -years risk for MI or coronary death >20%. The diseases cited by the National Cholesterol Education Program were:

- Diabetes mellitus
- Clinical coronary artery disease

- Symptomatic carotid artery disease
- Lower extremity artery disease
- Abdominal aortic aneurysm
- Chronic kidney Disease

2.13.9 Heart age

Also, called vascular age and defined as the age that corresponds to a person with normal risk factors and the same 10-year absolute risk. It is possible for a person to have a low 10-year risk but have a vascular age much older than their chronological age. For example a 40 year - old person with high levels of some risk factors may have the risk age of a 60-year old, because the risk equals that of a 60 year old with ideal risk factors levels (i.e. non-smoking, total cholesterol of 4mm/l and BP of12mmHg) (Cooney et al., 2012; D'Agostino Sr. et al., 2013).

2.14 Recent studies of prediabetic stage and coronary artery calcification

Nour and colleague (Nour et al, 2019) exhibited coronary artery calcium score (CACS) that has been shown to improve prediction of cardiovascular events beyond probabilistic risk scores in asymptomatic individuals and is recommended by guidelines to improve decision making. Patients undergoing CACS for risk prediction between June 2015 and March 2019. Patients were divided according to absence of diabetes (HbA1c <5.7%), prediabetes (HbA1c 5.7%-6.4%), or diabetes (HbA1c ≥ 6.5%) and followed for major adverse cardiovascular events [myocardial infarction, stroke, death (MACE) or coronary revascularization. Receiver operator characteristics were calculated for 10-year cardiovascular risk (by pooled cohort equation, PCE) and CACS for prediction of outcomes. A total of 6,394 subjects with HbA1c underwent CACS during the study period (1423 diabetes, 1751 prediabetes, and 3220 without diabetes). At a median follow-up of 1 year, there were 72 MACE and 200 MACE or revascularization events. Overall, CACS had similar discrimination of MACE by diabetes status (no diabetes: AUC 0.61 [0.52-0.70], prediabetes: AUC 0.70 [0.58-0.82], diabetes: AUC 0.54 [0.40-0.69]) and MACE or revascularization (no diabetes:

AUC 0.79 [0.74-0.84], prediabetes: AUC 0.79 [0.72-0.86], diabetes: AUC 0.78 [0.71-0.85]). CACS offered better discrimination of MACE and MACE or revascularization among patients with no diabetes, prediabetes and diabetes, table. Among patients with diabetes or prediabetes, CACS was associated with MACE (HR 1.65 [1.11-2.46] per standard deviation, P=0.013) or MACE or revascularization (HR 3.49 [2.81-4.35], P<0.001). In conclusion of this study, CACS is superior to PCE for prediction of MACE among asymptomatic patients including those with diabetes and pre-diabetes. These findings support the use of CACS over risk scores for cardiovascular risk stratification in these groups.

Milica and colleague (Milica et al, 2017) examined coronary artery calcium score (CACS) in 70 subjects with prediabetes. CAC score of 0 was present in 32 subjects. Minimal calcifications with a CAC score of 1–10 AU were present in 12 subjects with pre DM. moderate calcification of11–100 AU were present in 15 subjects. Nine subjects had significant calcifications with 101–400 AU. Two subjects had a CAC score of 100 AU which meant significant calcifications. Score risk below 2% was present in only 19 subjects, while a score risk of 15% and more was present in 23 subjects. No significant correlation was found between Score charts and CAC. However, a trend of finding more calcifications in those with a 10% and above Score risk was noted. In conclusion, An approach to risk assessment that combines the traditional risk factor-based paradigm with a more personalized atherosclerosis-imaging model may be appropriate for high risk individuals, such as subjects with pre diabetes.

Roberto and colleague (Roberto et al, 2016) examined HbA1c and CACS in 272 non diabetic patients aged between 40 and 70 years, with a normal fasting plasma glucose (FPG <5.6 mmol/L) and at least 1 CV risk factor. Prediabetes was defined as an HbA1c value of 5.7–6.4%. The result showed CAC score was higher in the prediabetes group compared to non-prediabetic subjects (131.7 ± 295.6 *vs.* 62.4 ± 178.8 AU, $p < 0.001$). Prediabetic subjects had higher mean carotid intimal media thickness (IMT) than non-exposed subjects (0.77 ± 0.14 *vs.* 0.61 ± 0.15 mm, $p < 0.001$). The proportion of prediabetic patients with CAC = 0 was significantly lower compared to non-exposed subjects (35% *vs.* 63%, $p < 0.01$). In contrast, the proportion of patients with a CAC >400 was significantly higher in the prediabetes group (10% *vs.* 3%, $p < 0.05$).

Moreover, carotid plaques were significantly more present in patients with prediabetes than in the normoglycemic subjects ($p < 0.01$). In a multiple linear regression, IMT was associated with HbA1c continuous levels ($p < 0.001$). In addition, logistic regression showed that higher HbA1c levels were associated with CAC and carotid plaques presence (p for trend for all < 0.001). In conclusion of the study, among patients with normal fasting glucose, HbA1c increase is associated with higher coronary and peripheral atherosclerotic burden in non-diabetic patients.

Monica and colleague (Monica et al, 2014) studied HbA1c and coronary artery disease in patient without diabetes mellitus whom undergoing coronary angiography between April 2007 to October 2012. There are 1,1703 patients, divided according to HbA1c tertiles (<5.5%, 5.5% - 5.79%, ≥5.8%). The result showed HbA1c was associated with gaining ($p < 0.001$); hypercholesterolemia ($p = 0.01$); renal failure ($p = 0.006$); hypertension ($p=0.002$), triglyceride ($p = 0.02$) and uric acid ($p = 0.04$). The HbA1c, but not fasting glycemia, was significantly associated with the prevalence of CAD (adjusted OR=1.51, 95% CI =1.15, 1.97, $p = 0.002$), with 5.8% identified by the receiver operating characteristic (ROC) curve as the best cut-off value for CAD prediction. HbA1c was significantly associated with carotid plaques prevalence.

2.15 Summarized Important Research Works related with Independent Variables

Table 2.14 Summarized Important Research Works related with Independent Variables

Researches / **Independent Variables**	Nour et a (2019)	Milica et al (2017)	Roberto et al (2016)	Ho and Shanahan (2016)	Evrard (2015)	Freedman et al (2015)	Zhang et al (2015)	Demer (2014)	Odink et al (2010)	Burke et al (2007)	**Total**
CT Coronary Artery Calcium Score (CACS)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**10**
Age	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**10**
Gender	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**10**
Hemoglobin A1c (HbA1c)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**10**
Prediabetes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**10**

Fasting blood sugar (FBS)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	**10**
Systolic Blood pressure	✓	✓	✓	✓				✓			**5**
Body Mass Index (BMI)	✓	✓	✓	✓				✓			**5**
Total cholesterol	✓	✓	✓	✓				✓			**5**
Triglyceride	✓	✓	✓	✓				✓			**5**
HDL	✓	✓	✓	✓				✓			**5**
LDL	✓	✓	✓	✓				✓			**5**
Smoking	✓	✓	✓	✓				✓			**5**

Summary

Coronary artery calcification is a well-recognized complication of atherosclerotic lesions in diabetic patients and prolongs hyperglycemia, particularly in prediabetic state, which correlates with increased plaque burden. Prolonged hyperglycemia contributes to the development of vessel calcification through multiple mechanisms including hyperglycemia-induced oxidative stress, endothelial dysfunction, renal function–induced alterations in mineral metabolism, increased phosphate circulation, increased inflammatory cytokine production, and release of osteoprogenitor cells from the marrow into the circulation. Detection type of coronary calcifications by combined imaging biomarkers, cell molecular biomarkers, substance biomarkers, and gene & protein biomarkers is useful in individual approach for prevention, treatment, or against progression of cardiovascular disease.

Chapter 3

Research Method

The research methods of the study, correlation between prediabetes (HbA1c 5.7 – 6.4% and fasting blood sugar less than 126 mg/dl), cardiovascular risk (FRS) and coronary artery calcium score (CACS), consist of components as follow;

3.1 Study design, data collection and population

3.2 Variables of the study

3.3 Conceptual framework

3.4 Research tool

3.5 Code data transferring

3.6 Data analysis and statistical used

3.1 Study design, data collection and population

This is a retrospective case study. The participants were identified by review medical records in PACS (Picture Archive and Communication System) and HIS (Hospital Information System) of Kasemrad International Hospital, Thailand, during Jan 2015 to December 2020.

Inclusion criteria were cases of CACS, during Jan 2015 to December 2020, who had HbA1c less than 6.5% and fasting blood sugar (FBS) less than 126 mg/dl, and had blood test of lipid profiles and uric acid.

Exclusion criteria were known case of ischemic heart disease, coronary balloon, coronary stent, diabetes, hypertension, autoimmune disease, gout, cancer, advanced

renal disease (GFR < 40 ml/minutes), liver disease, statin therapy, antihypertensive therapy, steroid therapy, or received bisphosphonates.

Participants were classified into two groups;

1) Non-diabetic group: HbA1c < 5.7% and FBS < 100 mg/dl
2) Pre-diabetic group: HbA1c 5.7 to 6.4% and FBS < 126 mg/dl

The CT coronary calcium (CAC) score was classified to four levels;

Level 1 CAC = 0	Very low risk for cardiovascular disease (CVD)
Level 2 CAC is 1-10	Low risk for cardiovascular disease (CVD)
Level 3 CAC is 11-100	Moderate risk for cardiovascular disease (CVD)
Level 4 CAC is 101-400	High risk for cardiovascular disease (CVD)
Level 5 CAC > 400	Very high risk for cardiovascular disease (CVD)

The percentage of Glycated hemoglobin (HbA1c) was classified to four intervals;

Class interval 1: HbA1C ≤ 5.4%

Class interval 2: 5.4% < HbA1c <5.7%

Class interval 3: 5.7% ≤ HbA1c <5.9%

Class interval 4: 5.9% ≤ HbA1c ≤ 6.4%

Framingham Risk Score (FRS) was classified to three categories;

Category 1: FRS ≤ 10%	Low risk for CVD
Category 2: 10% < FRS ≤ 19%	Intermediate risk for CVD
Category 3: FRS ≥ 20%	High risk for CVD

3.2 Variables of study

The study consists of two types of variables; twelve independent variables and a dependent variable, as seen on table 3.1

Table 3.1 Variables of the study

Type of variables	Variable (s)
Dependent variables	HbA1c, Age, Gender, BMI, and Fasting Blood Sugar (FBS) Systolic blood pressure (sys BP), Total cholesterol, Triglyceride, LDL, HDL, Smoking and Uric acid
A dependent variable	Coronary Artery Calcium Score (CACS)

3.3 Conceptual Framework

The study of correlation between prediabetic status, coronary artery calcification, and Framingham risk score, consist of independent variables (Age, Sex, BMI, FBS, HbA1c, systolic blood pressure, total cholesterol, Triglyceride, HDL, LDL, uric acid, and smoking) and a dependent variable (CACS). The relationship of variables was presented as conceptual framework (Figure 3.1)

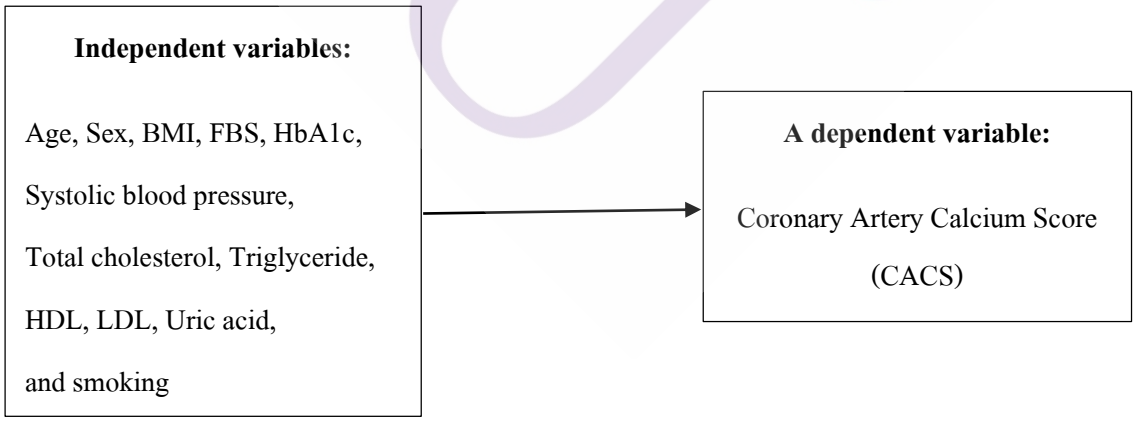


Figure 3.1 Conceptual framework

3.4 Research Tool

The study used Picture Archive and Communication System (PACS) and Hospital Information System (HIS) for analysis the retrieve patient's data, CT calcium score and CT chest, from Jan 2015 to September 2020.

3.5 Code Data Transferring

The study used a table form for coding variables (independent variables, dependent variables, and mediator variables), as follows table 3.2

Table 3.2 Coding for variables of the study

Variables of Data Reviewed	Code [0 /1]
1. Participants were classified into two groups;	
(1.1) Non-diabetic group: HbA1c < 5.7% and FBS < 100 mg/dl	E1.1 [….]
(1.2) Pre-diabetic group: 5.7 ≤ HbA1c ≤ 6.4% and FBS < 126 mg/dl	E 1.2 [….]
2. Presence or Absent of Coronary Artery Calcification (CAC)	
(2.1) Presence	E 2.1 [....]
(2.2) Absent	E 2.2 […]
3. Range of CAC Score	
(3.1) Level 1 CAC = 0	E 3.1 [....]
(3.2) Level 2 CAC is 1-10	E 3.2 […]
(3.3) Level 3 CAC is 11-100	E 3.3 [....]
(3.4) Level 4 CAC is 101-400	E 3.4 […]
(3.5) Level 5 CAC > 400	E 3.5 [....]
4. Percentage of HbA1c	
(4.1) Class interval 1: HbA1c ≤ 5.4%	E 4.1 [....]
(4.2) Class interval 2: 5.4% < HbA1c <5.7%	E 4.2 […]
(4.3) Class interval 3: 5.7% ≤ HbA1c <5.9%	E 4.3 [....]

(4.4) Class interval 4: 5.9% ≤ HbA1c ≤6.4%	E 4.4 […]
5. Framingham Risk Score by software calculated program	
(5.1) Category 1: FRS < 10% (Low risk)	E 5.1 [....]
(5.2) Category 2: 10% ≤ FRS ≤ 19% (Intermediate risk)	E 5.2 […]
(5.3) Category 3: FRS > 20% (High risk)	E 5.3 [....]

3.6 **Data analysis and statistical analysis**

3.6.1 Descriptive statistics are presented using mean and standard deviation (SD) for normally distributive variables: age, total cholesterol (mg/dl), HDL (mg/dl), systolic blood pressure (mmHg), LDL, and Uric acid.

3.6.2 The median and interquartile range (IQR) were used for non-normally distributed variables: Body Mass Index (BMI)

3.6.3 Statistical Analysis between risk factors of FRS and coronary calcification using T-test in age, total cholesterol, HDL, and systolic blood pressure.

3.6.4 Chi-square test was used in smoking.

3.6.5 Fisher's exact test was used in gender (male/female)

3.6.6 Mann-Whitney test was used in BMI.

3.6.7 The Chi-square test was performed to assess the difference between the distribution of CAC score in nondiabetic group and prediabetic group.

3.6.8 The Chi-square test and Cramer's V were performed to measure association between CAC levels and HbA1c class intervals, and between CAC levels and FRS categories.

3.6.9 Logistic Regression model was used to estimate OR and 95% CI in relationship between HbA1c intervals and number of participants with presence of coronary calcification.

The summary of statical used for data presentation and data analysis for each variables and correlation between variables was presented on table 3.3

Table 3.3 Statistical used analysis for variables and correlation between variables.

Variables / Correlation between variables	Statistical Used Analysis
Age, Total cholesterol (mg/dl), HDL (mg/dl), Systolic blood pressure (mmHg), LDL, Triglyceride, and Uric acid.	Standard deviation (SD)
Body Mass Index (BMI)	The median and interquartile range (IQR)
Correlation between Smoking and CACS	Chi-square test
Correlation between Risk factors of FRS (systolic BP, HDL, and Total Cholesterol) and Coronary Artery Calcification (CAC)	T-test
Difference between the distribution of CAC score (CACS) in nondiabetic group and prediabetic group.	The Chi-square test
Correlation between CACS and HbA1c.	The Chi-square test and Cramer's V
Correlation between CACS and FRS	The Chi-square test and Cramer's V
Estimated OR and 95% CI in relationship between HbA1c intervals and number of participants with presence of coronary calcification (CAC)	Logistic Regression model
Estimated OR and 95% CI in relationship between CACS intervals and number of pre-diabetic participants	Logistic Regression model
Estimated OR and 95% CI in relationship between CACS intervals and number of Non-diabetic participants	Logistic Regression model

Chapter 4

Results

The research is a retrospective case control study. The participants, 2,313 cases with CT coronary artery calcium score (CACS), were identified by review medical records in PACS (Picture Archive and Communication System) and HIS (Hospital Information System) of Kasemrad International Hospital, Thailand, during Jan 2015 to October 2020.

674 cases were excluded from study, due to statin therapy (224 cases), known diabetes (114 cases), known hypertension (96 cases), known co-existing diabetes and dyslipidemia (111 cases), known co-existing diabetes and hypertension (86 cases), known co-existing diabetes, hypertension, and dyslipidemia (n = 37cases), and known cardiovascular disease (6 cases)

1,639 cases were included in the research, as seen along flow chart figure 4.1, 4.2 and 4.3. The SPSS and Excel statistic program were used for calculation and assessment the variables, as presented with percentage, standard deviation (S.D), The median and interquartile range (IQR), Chi-square test, T-test, and Logistic Regression.

The results show 1,639 cases divided two groups; (1) prediabetic group, 756 cases and (2) non-diabetes group, 883 cases. Genders in each group (figure 4.3), consist of prediabetic group (n= 756, male = 416, 55%, female = 340, 45%) and nondiabetic group (n = 883, male = 399, 46%, female = 484, 54%).

The mean age of prediabetes group (54.84 years) is elder than non-diabetes group (44.8 years). There are seven variables with significant difference between participants groups, consist of age, HbA1c, fasting blood sugar, total cholesterol, LDL, BMI, and serum uric acid.

, as seen on table 4.1

The most age range of prediabetes group is 50 to 54 years (246 cases) and then 55 to59 years (166 cases), respectively. While, the most age range of non-diabetes group is 40 to 44 years (244 cases) and then 45 to 49 years (236 cases). Most of prediabetes participants (456 cases, 61%) had intermediate cardiovascular risk score (Framingham Risk Score: FRS). While, most of non-diabetes participants (665 cases, 76%) had low risk of FRS.

There was a significant correlation between diabetic status (prediabetes and non-diabetes) and presence of coronary artery calcification, with adjusted Odds Ratio = 2.38 [95% CI (1.98– 14.46)]. That means in cases of prediabetes had 2.38 times to presence of coronary artery calcification, seen on table 4.5.

Sixty three percent (446 cases) of participants with positive coronary artery calcification (CACS >0) were prediabetes (5.9% < HbA1c ≤6.4%). In the contrast, seventy percent of participants with negative coronary artery calcification (CACS = 0) were non-diabetes, table 4.6. Most of CACS range in prediabetes group was 11-100 (272 cases, 36%). Most of CACS in non-diabetes group was zero (643 cases, 73%). In overall, the most of CACS in participants was zero (826 cases, 51%), and then CACS 11-100 (368 cases, 23%), seen on table 4.7

Multiple logistic regression indicated that positive CACS associated with higher HbA1c (p for trend < 0.001) and these associations were independent after adjustment for age, sex, smoking, systolic blood pressure, HDL, cholesterol, LDL, triglyceride and uric acid, seen on table 4.8

Multiple logistic regression indicated trend association between cardiovascular risk (FRS) and coronary artery calcium score (CACS), after adjustment for HbA1c, LDL, triglyceride and uric acid. The prediabetes had trends of increased CACS among intermediate to high cardiovascular risk (FRS), seen on table 4.9

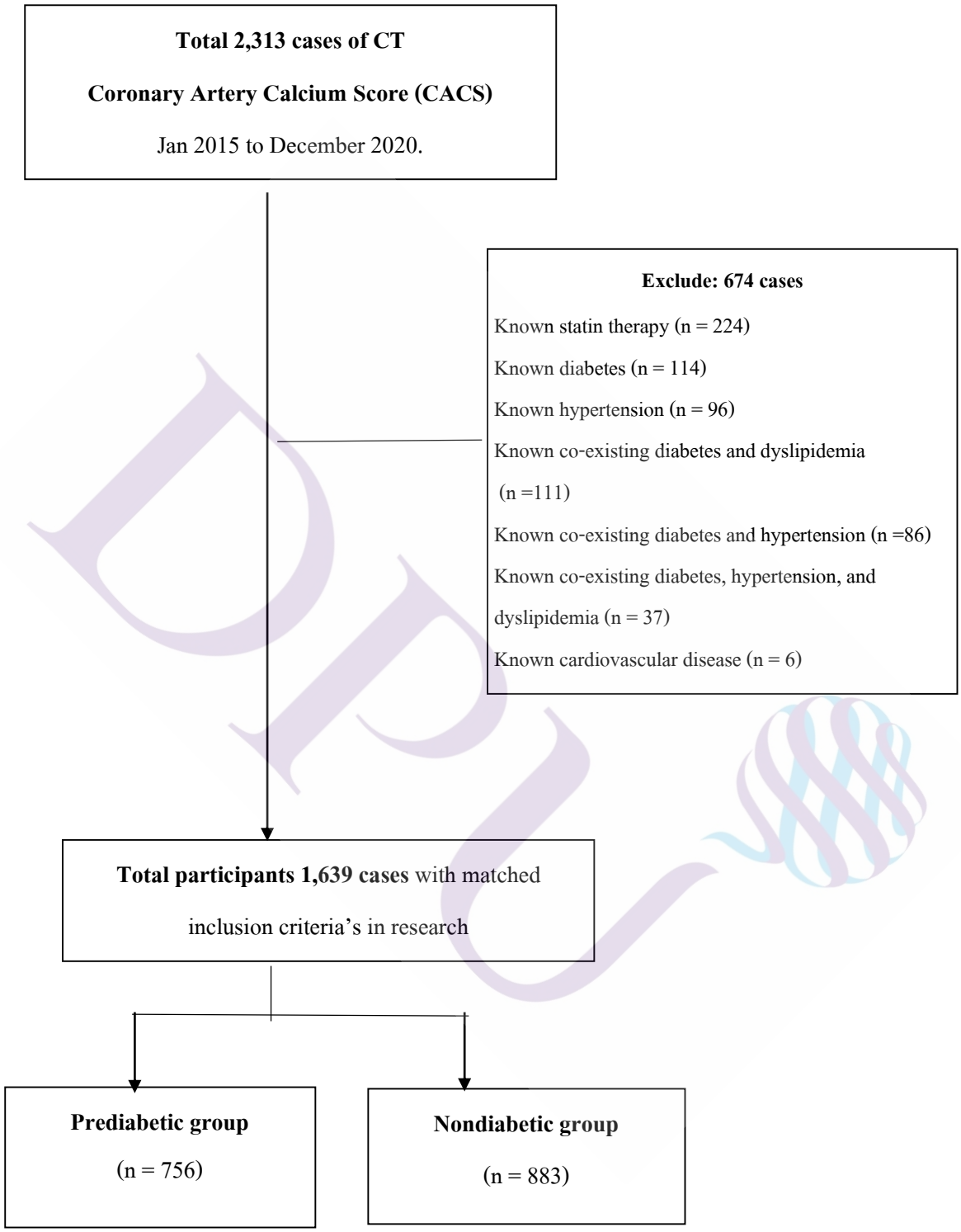


Figure 4.1 Flowchart of study design and selection criteria

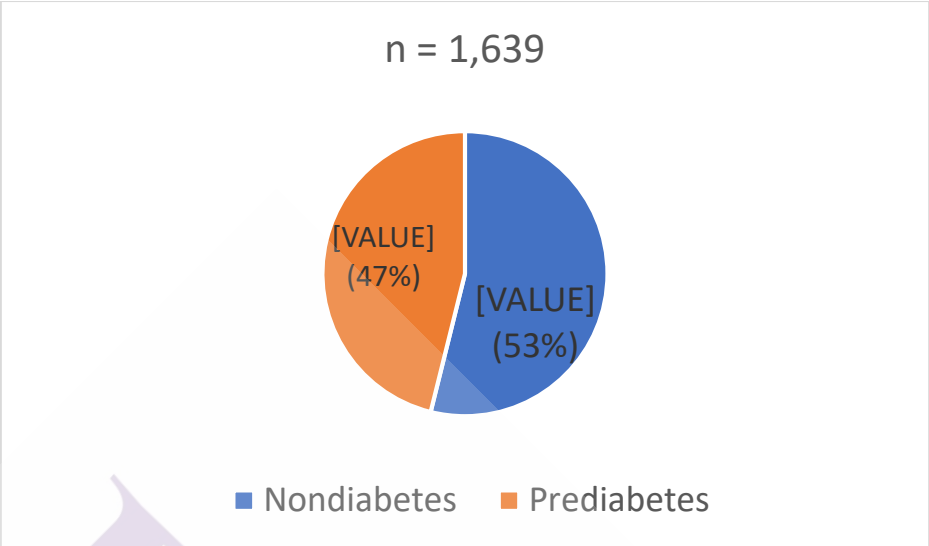


Figure 4.2 Pie chart represented two groups of study, prediabetic group (n= 756, 47%) and nondiabetic group (n = 883, 53%)

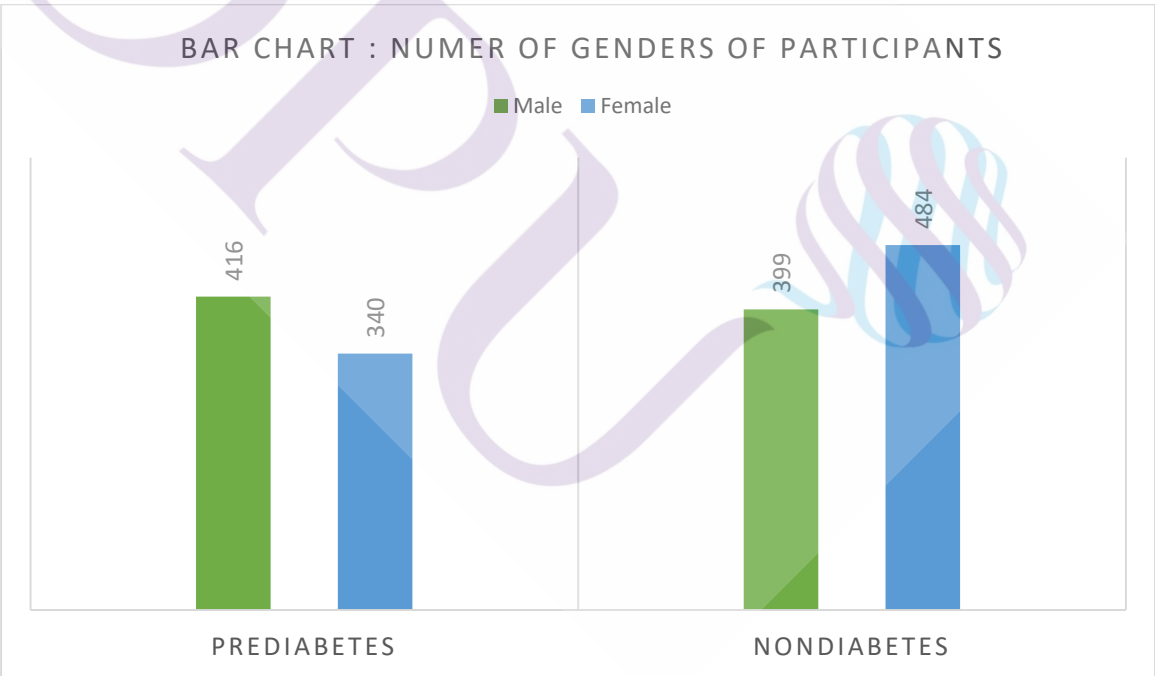


Figure 4.3 Bar chart represented number of genders in each group of the study, prediabetic group (n= 756, male = 416, 55%, female = 340, 45%) and nondiabetic group (n = 883, male = 399, 46%, female = 484, 54%)

Table 4.1 Baseline characteristics of the study participants

Demographic Characteristics	Prediabetes (n = 756)	Nondiabetes (n = 883)	P-value
Age, mean (SD)	54.84 ± 6.87	44.82 ± 7.83	0.003*
Male (%) / Female (%)	416 (55%) / 340 (45%)	399 (46%) / 484 (54%)	0.47
HbA1c, mean (range)	6.2 (5.7 – 6.4%)	5.1 (4.8 - 5.6%)	< 0.001*
Fasting Blood Sugar (mg/dl), mean (range)	112 (100 – 124)	91 (86 - 99)	< .0001*
History of smoking, n (%)	98 (13%)	101 (12%)	0.45
Total Cholesterol (mg/dl), mean (SD)	199.50 (36.7)	158.40 (34.9)	0.012*
HDL (mg/dl), mean (SD)	48.3 (13.6)	51.4 (10.2)	0.15
LDL (mg/dl), mean (SD)	142 (14.8)	98 (12.8)	0.023*
Triglyceride (mg/dl), mean (SD)	144 (28.8)	136 (26.4)	0.14
Systolic Blood Pressure (mmHg), mean (SD)	128 (8.6)	118 (6.8)	0.51
BMI (Kg/m2), median (IQR)	27.9 (7.3)	21.6 (5.2)	0.036*
Uric acid (mg/dl), mean (SD)	5.28(1.38)	3.82 (1.13)	<0.001*

*P-value < 0.05 significant

From table 4.1, the mean age of prediabetes group was elder than non-diabetes group, mean age = 54.84 years and 44.8 years, respectively. There were seven variables with significant difference between participants groups, consist of age, HbA1c, fasting blood sugar, total cholesterol, LDL, BMI, and serum uric acid.

Table 4.2 Age ranges of two groups participants

Age (years)	Pre-diabetes (HbA1c 5.7 to 6.4%) n (%)	Non-diabetes HbA1c < 5.7% n (%)	Total
30-34	14 (1.85%)	88 (10%)	102
35-39	18 (1.05%)	98 (11%)	116
40-44	55 (8%)	244 (28%)	299
45-49	117 (16%)	236 (27%)	353
50-54	246 (33%)	84 (10%)	330
55-59	166 (22%)	43 (5%)	209
60-64	92 (13%)	46 (6%)	138
65-69	28 (4%)	34 (4%)	62
70-74	18 (3%)	9 (1%)	27
75 +	2 (0.26%)	1 (0.11%)	3
Total	**756**	**883**	**1,639**

From table 4.2, the most of participant's age range in prediabetes group was 50 to 54 years (246 cases) and then 55 to59 years (166 cases), respectively. For the most participants age range in non-diabetes group was 40 to 44 years (244 cases) and then 45 to 49 years (236 cases).

Table 4.3 Characteristics of the participants, using factors of Framingham Risk Score (FRS)

Demographic Characteristics	Prediabetes (n = 756)	Non-diabetes (n = 883)	P-value
Age, mean (SD)	54.84 ± 6.87	44.8 ± 7.827	0.003*
Gender Male (%) / Female (%)	416 (55%) / 340 (45%)	399 (46%) / 484 (54%)	0.47
HbA1c, mean (range)	6.2 (5.7 – 6.4%)	5.1 (4.8 - 5.6%)	< 0.001*
Fasting Blood Sugar (mg/dl), mean (range)	112 (100 – 124)	91 (86 - 99)	< .0001*
History of smoking, n (%)	98 (13%)	101 (12%)	0.45
Total Cholesterol (mg/dl), mean (SD)	199.50 (36.7)	158.40 (34.9)	0.012*
HDL (mg/dl), mean (SD)	48.3 (13.6)	51.4 (10.2)	0.15
Systolic Blood Pressure (mmHg), mean (SD)	128 (8.6)	118 (6.8)	0.51

From table 4.3, the table represent only risk factors for cardiovascular event, according to Framingham Risk Score (FRS), consist of age, gender, diabetes status (HbA1c or FBS), smoking, total cholesterol, HDL, and systolic blood pressure. There were four variables with significant difference between study groups, consist of age, HbA1c, fasting blood sugar (FBS), and total cholesterol.

Table 4.4 Percentage of Cardiovascular Risk by Framingham Risk Score (FRS) in pre-diabetes and non-diabetes

Framingham Risk Score (FRS)	Pre-diabetes n (%)	Non-diabetes n (%)	Total
Low Risk (< 10%)	278 (30%)	665 (70%)	**943**
Intermediate Risk (10-19%)	456 (69%)	211(31%)	**667**
High Risk (≥20%)	22 (76%)	7 (24%)	**29**
Total	**756**	**883**	**1,639**

From table 4.4, most of prediabetes participants (456 cases, 61%) had intermediate risk score of FRS. While, most of non-diabetes participants (665 cases, 76%) had low risk of FRS.

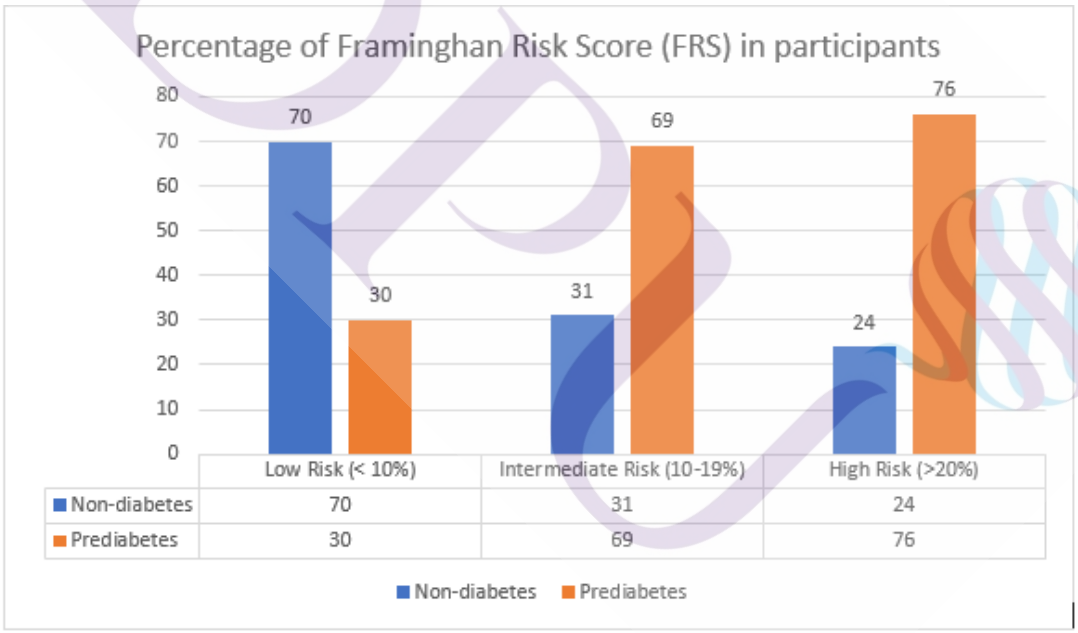


Figure 4.4 Percentage of Cardiovascular Risk by Framingham Risk Score (FRS) in pre-diabetes and non-diabetes

Table 4.5 Relation of diabetes status and coronary artery calcification

	CAC > 0 cases (n)	**CAC = 0 cases (n)**	**Total**
Pre-diabetes HbA1c 5.7 to 6.4%	573	183	**756**
Non-diabetes HbA1c < 5.7%	140	743	**883**
Total	**713**	**926**	**1,639**

Adjusted Odds Ratio (OR) = 2.38886 [95% CI (1.98212 – 14.9838)]

From table 4.5, the table showed correlation between diabetic status (prediabetes and non-diabetes). There was a significant correlation between diabetic status (prediabetes and non-diabetes) and presence of coronary artery calcification, with adjusted Odds Ratio = 2.38 [95% CI (1.98– 14.46)]. That means in cases of prediabetes had 2.38 times to presence of coronary artery calcification.

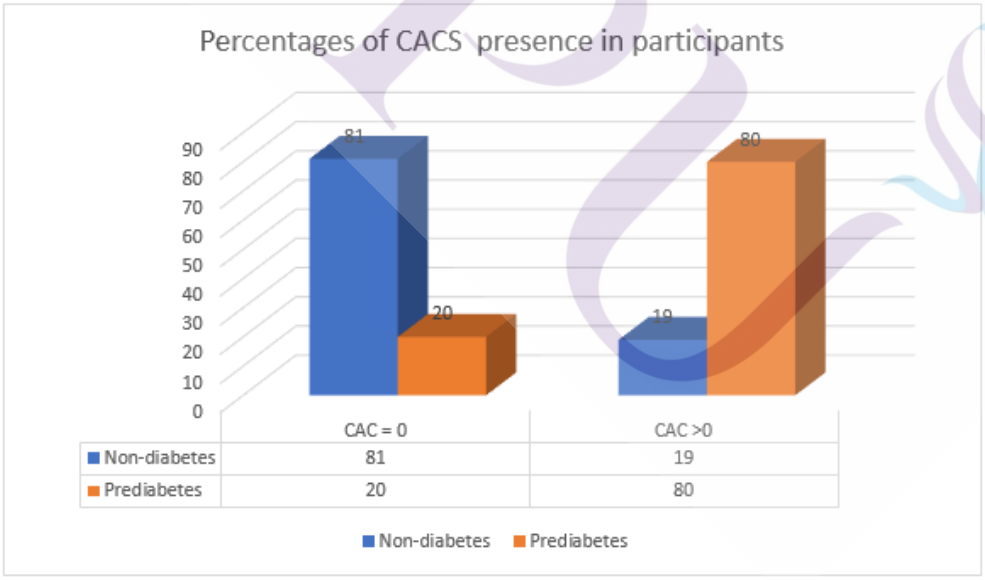


Figure 4.5 Bar chart: percentage of presence or absence coronary artery calcification in two groups of participants

Table 4.6 Relation of HbA1c and coronary artery calcification (CAC)

	CACS > 0 n (%)	**CACS = 0 n (%)**	**P value**
5.9% ≤ HbA1c ≤6.4%	446 (62.5%)	85 (9.17%)	**0.008***
5.7% ≤ HbA1c <5.9%	127 (17.8%)	98 (10.5%)	**0.855**
5.4% < HbA1c <5.7%	74 (10.3%)	644 (69.5%)	**0.014***
HbA1c ≤ 5.4%	66 (9.2%)	99 (10.7%)	**0.450**
Total (n)	713 (100%)	926 (100%)	

From table 4.6, most of prediabetic participants, 446 cases (62.5%), 5.9% ≤ HbA1c ≤6.4%, had significant associated with positive coronary artery calcification (CACS >0). In the contrast, most of nondiabetics participants, 644 cases (69.5%), 5.4% < HbA1c <5.7%, had significant associated with negative coronary artery calcification (CACS = 0).

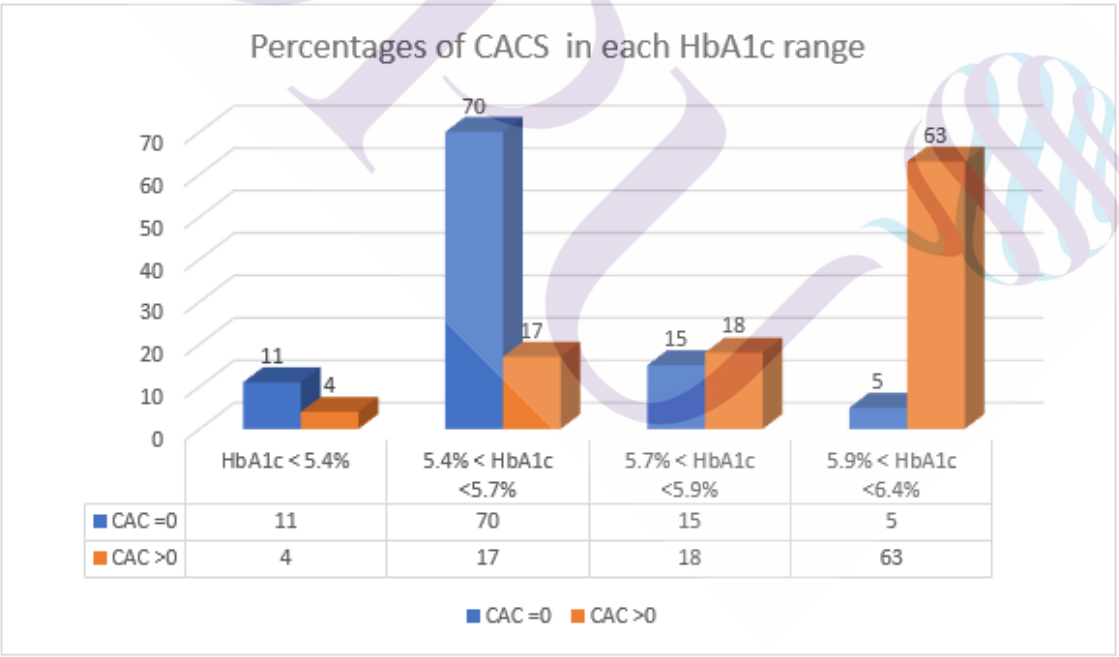


Figure 4.6 Bar chart:: percentage of coronary calcification score (CACS) in each HbA1c range

Table 4.7 Percentage of CACS in pre-diabetes and non-diabetes

CACS	Pre-diabetes cases (n, %)	Non-diabetes cases (n, %)	P value
CACS = 0	183 (24.2%)	743 (84.1%)	**0.016***
CACS 1 -10	174 (23.0%)	84 (9.5%)	**0.367**
CACS 11-100	272 (35.9%)	54 (6.1%)	**0.013***
CACS 101-400	118 (15.6%)	2 (0.2%)	**0.002***
CACS > 400	9 (1.2%)	0	**< 0.001***
Total	**756**	**883**	**1,639**

Table 4.7 showed classifications of coronary artery calcium score (CACS) associated with numbers of participants (prediabetes and non-diabetes). Most of CACS range in prediabetes group was 11-100 (272 cases, 36%) with significant [P =0.013]. Most of CACS in non-diabetes group was zero (643 cases, 73%) with significant [P = 0.016].

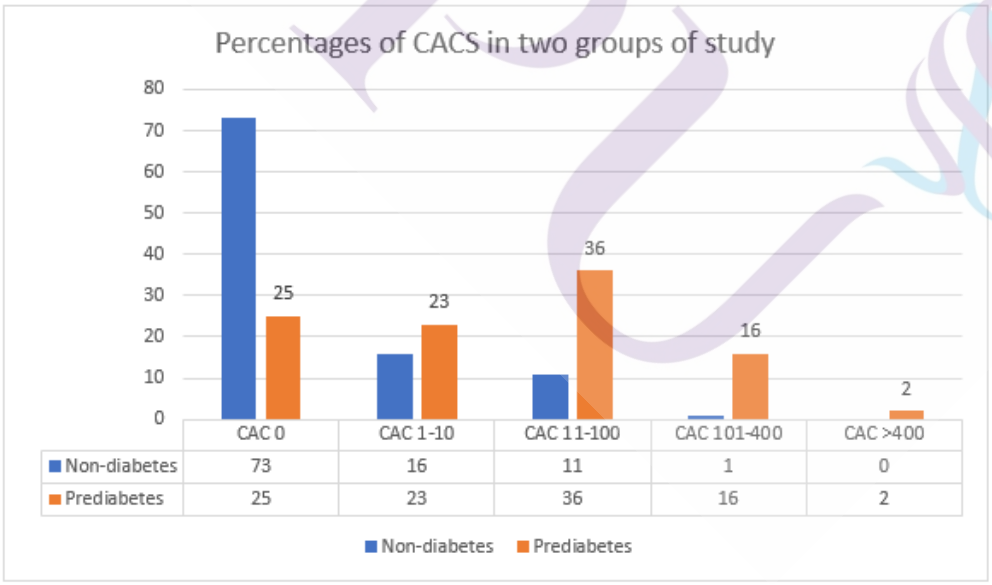


Figure 4.7.1 Bar chart: percentage of coronary calcification in two groups of participants

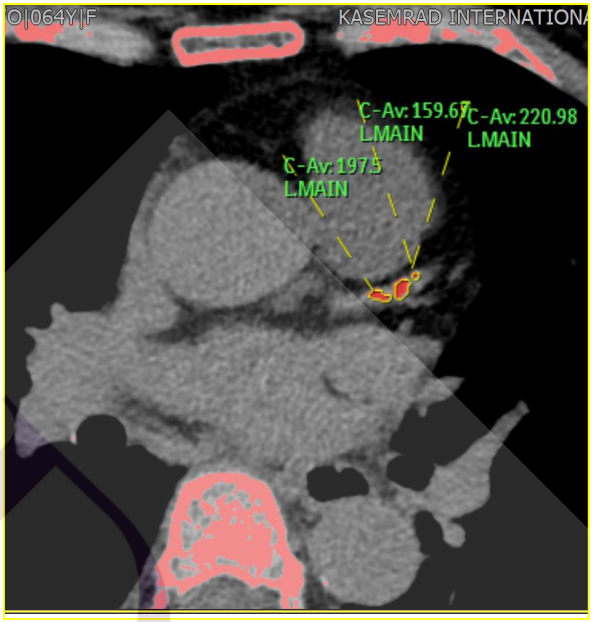


Figure 4.7.2 CT coronary artery calcium score (CACS) in a 64-year-old female, HbA1c 5.9% with moderate cardiovascular risk (FRS = 13%), CACS = 202.3

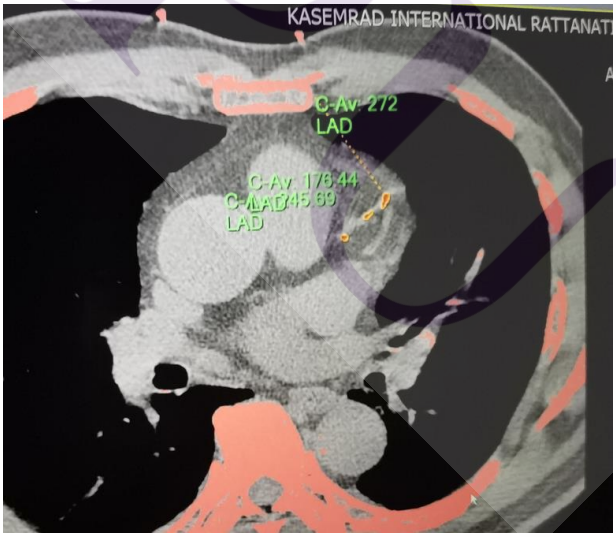


Figure 4.7.3 CT coronary artery calcium score (CACS) in a 56-year-old female, HbA1c 6.2% with moderate cardiovascular risk (FRS = 12%), smoking, CACS = 186.4

Table 4.8 Logistic regression of positive coronary artery calcification score (CACS > 0) in study participants divided into HbA1c quartiles

	No. of participants with positive CACS > 0	**Multivariate OR (95% CI) Logistic regression model***
5.9% ≤ HbA1c ≤6.4%	446 (62.5%)	8.14 (3.56 -18.44)
5.7% ≤ HbA1c <5.9%	127 (17.8%)	5.76 (2.42-14.36)
5.4% < HbA1c <5.7%	74 (10.3%)	2.70 (1.39- 8.65)
HbA1c ≤ 5.4%	66 (9.2%)	1.00 (reference)
P for trend		< 0.001

*Logistic regression model was used to estimate OR and 95% CI

Model: adjustment for age, sex, BMI, smoking, systolic blood pressure, HDL, cholesterol, LDL, triglyceride and uric acid.

From table 4.8, multiple logistic regression indicated that positive CACS associated with higher HbA1c (p for trend < 0.001) and theses associations were independent after adjustment for age, sex, smoking, systolic blood pressure, HDL, cholesterol, LDL, triglyceride and uric acid.

Table 4.9 Logistic regression model of positive coronary artery calcification score (CACS > 0) in participants divided into Framingham Risk Score (FRS)

Participants with Framingham Risk Score (FRS)	No. of participants with positive CACS > 0	Multivariate OR (95% CI)
Prediabetes		
- Low Risk (< 10%)	216	1.00(reference)
- Intermediate Risk (10-19%)	339	2.36 (1.06 – 5.46)
- High Risk (≥20%)	18	8.64 (2.65 – 18.58)
Non-diabetes		
- Low Risk (< 10%)	16	1.00(reference)
- Intermediate Risk (10-19%)	121	7.86 (3.08-18.18)
- High Risk (≥20%)	3	3.70 (1.59- 8.65)
P for trend		**< 0.001**

*Logistic regression model was used to estimate OR and 95% CI

Model: adjustment for BMI, LDL, triglyceride, and uric acid.

From table 4.9, multiple logistic regression indicated trend association between cardiovascular risk (FRS) and coronary artery calcium score (CACS), after adjustment for HbA1c, LDL, triglyceride and uric acid. The prediabetes had trends of increased CACS among intermediate to high cardiovascular risk (FRS)

In summary, the study was concluded significant results as follows;

(1) Prediabetic status (5.7% ≤ HbA1c ≤ 6.4%) significantly associated with coronary calcification about 2.38 times to non-diabetic status (Adjusted Odds Ratio = 2.38886 [95% CI (1.98212 – 14.983800)]

(2) Intermediate cardiovascular risk (FRS) associated with positive coronary artery calcification about 2.36 times to low cardiovascular risk [Multivariate adjusted OR = 2.36 (95% CI (1.06 – 5.46)]

(3) High cardiovascular risk (FRS) associated with positive coronary artery calcification about 8.64 times to low cardiovascular risk [Multivariate adjusted OR = 8.64 (95% CI (2.65 – 18.58)]

(4) The mean age of prediabetes group, 54.84 $\pm$ 6.87 years, was elder than non-diabetes group, 44.82 $\pm$ 7.83 years, (P-value = 0.003).

(5) BMI of prediabetic group, average = 27.9 kg/m2, was significantly higher than non-diabetic group, average = 21.6 kg/m2, (P-value = 0.036).

(6) Total cholesterol and LDL of prediabetic group, 199.50 $\pm$ 36.7mg/dl and 142 $\pm$ 14.8 mg/dl, were significantly higher than non-diabetic group, 158.40 $\pm$ 34.9mg/dl and 98 $\pm$ 12.8mg/dl, P-value = 0.012 and 0.023, respectively.

(7) Serum uric acid in prediabetic group, 5.28 $\pm$ 1.38 mg/dl, was significantly higher than non-diabetic group, 3.82 $\pm$ 1.13 mg/dl, P-value < 0.001.

Chapter 5

Conclusion Discussion and Suggestion

The research is a retrospective case control study. The participants, 2,313 cases with CT coronary artery calcium score (CACS), were identified by review medical records in PACS (Picture Archive and Communication System) and HIS (Hospital Information System) of Kasemrad International Hospital, Thailand, during Jan 2015 to December 2020.

1,639 cases were included in the research, divided two groups; (1) prediabetic group, 756 cases and (2) non-diabetes group, 883 cases. Genders in each group consist of prediabetic group (n= 756, male = 416, 55%, female = 340, 45%) and nondiabetic group (n = 883, male = 399, 46%, female = 484, 54%).

The mean age of prediabetes group, 54.84 $\pm$ 6.87 years, was elder than non-diabetes group, 44.82 $\pm$ 7.83 years, (P-value = 0.003). BMI of prediabetic group, average = 27.9 kg/m2, was significantly higher than non-diabetic group, average = 21.6 kg/m2, (P-value = 0.036). Total cholesterol and LDL of prediabetic group, 199.50 $\pm$ 36.7mg/dl and 142 $\pm$ 14.8 mg/dl, were significantly higher than non-diabetic group, 158.40 $\pm$ 34.9mg/dl and 98 $\pm$ 12.8mg/dl, P-value = 0.012 and 0.023, respectively. Serum uric acid in prediabetic group, 5.28 $\pm$ 1.38 mg/dl, was significantly higher than non-diabetic group, 3.82 $\pm$ 1.13 mg/dl, P-value < 0.001.

The results of the study that had statistically significant under hypothesis were concluded as follows;

(8) Prediabetic status (5.7% ≤ HbA1c ≤ 6.4%) significantly associated with coronary calcification about 2.38 times to non-diabetic status (Adjusted Odds Ratio = 2.38886 [95% CI (1.98212 – 14.983800)]

(9) Intermediate cardiovascular risk (FRS) associated with positive coronary artery calcification about 2.36 times to low cardiovascular risk [Multivariate adjusted OR = 2.36 (95% CI (1.06 – 5.46)]

(10) High cardiovascular risk (FRS) associated with positive coronary artery calcification about 8.64 times to low cardiovascular risk [Multivariate adjusted OR = 8.64 (95% CI (2.65 – 18.58)]

Hypothesis Testing

a. Is there a statistically significant relationship between CAC and HbA1c in prediabetic cases?

b. Is there a statistically significant relationship between CAC and cardiovascular risk factors (determined by FRS) in prediabetic cases?

The result of hypothesis testing for (a) was accepted. There was statistically significant relationship between CAC and HbA1c in prediabetic cases. Prediabetic status (5.7% ≤ HbA1c ≤ 6.4%) significantly associated with coronary calcification about 2.38 times to non-diabetic status (Adjusted Odds Ratio = 2.38886 [95% CI (1.98212 – 14.983800)].

The result of hypothesis testing for (b) was accepted. There was statistically significant relationship between CAC and cardiovascular risk factors (determined by FRS) in prediabetic cases. Intermediate and high cardiovascular risk (FRS) associated with positive coronary artery calcium score (CACS) [Multivariate adjusted OR = 2.36 (95% CI (1.06 – 5.46)) and Multivariate adjusted OR = 8.64 (95% CI (2.65 – 18.58)).

In addition, the results of study showed total cholesterol and LDL of prediabetic group, 199.50 $\pm$ 36.7mg/dl and 142 $\pm$ 14.8 mg/dl, were significantly higher than non-diabetic group, 158.40 $\pm$ 34.9mg/dl and 98 $\pm$ 12.8mg/dl, P-value = 0.012 and 0.023, respectively.

An interesting result, the serum uric acid in prediabetic group, 5.28 $\pm$ 1.38 mg/dl, was significantly higher than non-diabetic group, 3.82 $\pm$ 1.13 mg/dl, P-value < 0.001.

Discussion

The significant results of 5-year retrospective case control study which analyzed correlation between prediabetic status, HbA1c 5.7 to 6.4%, cardiovascular risk determined by Framingham Risk Score (FRS) and coronary artery calcium score (CACS), can discussed as six related topics as follows;

1. Aging and prediabetic status
2. Aging and positive coronary artery calcium (CAC)
3. Prediabetes and coronary artery calcium (CAC)
4. Framingham risk score (FRS) and positive CAC score (CACS)
5. Dyslipidemia and positive CAC
6. Serum uric acid and cardiovascular disease

1. Aging and prediabetic status

Aging, especially menopause, is an indicator of the transition from reproductive to non-reproductive life and is associated with biological and hormonal changes. The risk is related to post menopause is due to a sudden decrease of estrogen hormone, which has protective effects on lipid, glycemic metabolism and vessels (Rossi et al., 2002). Menopausal status and estrogen deficiency were frequently associated with increase in BMI, insulin-resistance, sodium retention, and with increased smooth muscle cell proliferation leading to an increase in systemic vascular resistance, hypertension, atherosclerosis, coronary calcification, prediabetes, diabetes, and obesity.

In the swedish national study on aging and care (Y. Shange et at, 2019) showed 2575 diabetes free participants aged ≥ 60 years that were examined at baseline and followed for up to 12 years. The results of study showed 918 (36%) individuals had prediabetes. Of them, 204 (22%) reverted to normoglycemia (3.4/100 person/years, 95% CI 5.6–12.3), 119 (13%) developed diabetes (2.0/100 person/years, 95% CI 1.7–2.4) and 215 (23%) died (13.0/100 person/years, 95% CI 11.4–14.9) during 12 years follow up. The rates of reversion, progression and mortality were higher in the first 6 years than in the second 6 years follow up, albeit not statistically significant. Lower systolic blood pressure, absence of heart diseases and weight loss promoted the reversion from prediabetes to normoglycemia, while obesity condition accelerated progression to diabetes, seen on Figure 5.1.

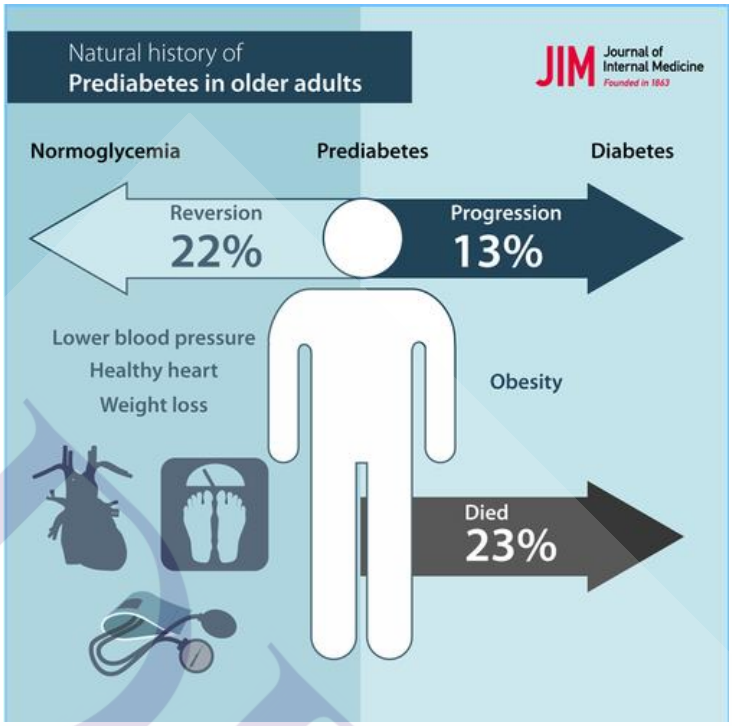


Figure 5.1: Natural history of prediabetes in older adults.

Source: Shang Y (2019)

In conclusion, aging is an indicator of the transition from reproductive to non-reproductive life and is associated with biological and hormonal changes, leading to insulin resistance and prediabetic status.

2. Aging and positive coronary artery calcium (CAC)

By 2030, about 20% of the population will be aged > 65 years. In this age group CVD will result in 40% of all deaths and will be the leading cause (North & Sinclair, 2012). The cardiovascular system is strongly affected by the ageing process leading to progressive deterioration in structure and function of the heart, coronary artery and vasculature that contribute to the development of atherosclerosis and CVD (Costa et al., 2015). Epidemiologic studies revealed that at any age the risk of cardiovascular events varies widely (4-5-fold) depending on the associated risk factors. Studies indicate that the chances of surviving to age 85 years have decreased significantly with cumulative risk factors, from 37% for men without risk factors to 2% with five risk factors and from 65% for women without risk factors to 14% with five risk factors (Kannel & Vasan, 2009).

Heart age, or called vascular age, defined as the age that corresponds to a person with normal risk factors and the same 10-year absolute risk. It is possible for a person to have a low 10-year risk but have a vascular age much older than their chronological age. For example, a 40-year-old person with high levels of some risk factors may have the risk age of a 60-year-old, because the risk equals that of a 60-year-old with ideal risk factors levels (i.e., non-smoking, total cholesterol of 4mm/l and BP of 12mmHg) (Cooney et al., 2012; D'Agostino Sr. et al., 2013).

In conclusion, chronological aging associated with progression of cardiovascular age, atherosclerosis, vascular calcification and coronary calcification, especially in risk conditions such as smoking, hypertension, diabetes and dyslipidemia.

3. Prediabetes and positive CACS

The research of Nishikawa and colleague (Nishikawa et at, 2000) is the one of the most significant discoveries in patients with diabetes mellitus that unraveling the nature of hyperglycemic damage mainly driven by the accumulation of free radicals, namely, superoxide anion, which is capable of activating an array of cellular pathways including polyol and hexosamine flux, advanced glycation end products (AGEs), PKC, and nuclear factor-κB–mediated vascular inflammation. The results shown increased levels of glucose and other reducing sugars such as galactose and fructose reacted amino groups of proteins to form Schiff bases to yield AGEs. The interaction of AGEs with receptors for advanced glycation end production (RAGEs) activates PKC-ζ to trigger downstream activation of signaling through p38 mitogen activated PK, transforming growth factor-β, and nuclear factor-κB. Significant data support that AGE treatment of VSMC promotes calcification through multiple mechanisms including increasing levels of alkaline phosphatase (ALP), a bone matrix protein, decreased expression of VSMCs markers, and increased expression of Runt-related transcription factor-2 (Runx2), suggesting RAGE promotes transformation of VSMCs into osteoblast-like phenotype.

Kay, Simpson and Stewart (2016) studied AGE/RAGE signaling, the results showed AGE/RAGE signaling exacerbates oxidative stress though a feed-forward loop. AGE activation results in the increased production of reactive oxygen species by stimulating specific signaling cascades such as transforming growth factor-β, nuclear factor-κB, and Nox-1. SMC expression of S100A12, a human RAGE ligand, increased medial calcification in proximal aorta and innominate arteries of ApoE mice, which was associated with increases in BMP-2 and Runx2.

The actions of S100A12 were dependent on RAGE and oxidative stress signaling because both recombinant soluble RAGE (decoy receptor for RAGE) and NAD(P)H oxidase (Nox) inhibition reduced osteogenic programming and calcification. Ligands for RAGE are quenched by the soluble form of the receptor, and serum levels of this decoy receptor in hemodialysis patients are inversely associated with vascular calcification. In each of these experiments, although AGEs have been shown to induce vascular calcification, the specific links to diabetes mellitus remain somewhat elusive.

Hyperglycemia itself also increases oxidative stress by increasing glucose oxidation in the citric acid cycle. Generation of mitochondrial reactive oxygen species (ROS) is an important contributing factor, as treatment with a mitochondrial uncoupler prevents upregulation of reactive oxygen species. When glucose levels are elevated, the polyol pathway converts glucose to sorbitol using NADPH (nicotinamide adenine dinucleotide phosphate hydrogen) as a cofactor. As a result, the antioxidant glutathione, which also uses NADPH as a cofactor, becomes dysfunctional decreasing cellular resistance to oxidative stress. Many groups have shown that oxidative stress powerfully upregulates Runx2 and promotes VSMC calcification. Oxidative stress and oxidized lipids also induce receptor activator of nuclear factor-κB ligand in mouse VSMCs via Runx2. Mice deficient in a decoy receptor for receptor activator of nuclear factor-κB ligand, osteoprotegerin, develop extensive vascular calcification which is reduced by osteoprotegerin treatment (Bucay et al, 1998; Panizo et al, 2009).

Hyperglycemia also can activate the PKC pathway by increasing the synthesis of diacylglycerol that plays a critical role in activating PKC, PKCβ, PKCδ, and PKCα. Globally, genes involved in vessel dilation such as nitric oxide are decreased, whereas those involved in vessel constriction such as endothelin-1 are increased. Basic calcium phosphate crystals deposit in atherosclerotic lesions and colocalize with inflammatory macrophages.

Nadra and colleague (Nadra et al, 2005) showed that ingestion of basic calcium phosphate in macrophages triggers a proinflammatory response, including secretion of the inflammatory cytokines tumor necrosis factor-α, interleukin-1, and interleukin-8. PKCα was a key mediator of these effects. Others have suggested tumor necrosis factor–induced nuclear factor-κB can promote inorganic phosphate-induced calcification of human aortic SMCs while suppressing pyrophosphate (inhibitor of calcification). Thus, activation of PKC by hyperglycemia might produce a vicious cycle whereby ingestion of basic calcium phosphates by macrophages not only induces inflammation but also promotes calcification.

Glucose metabolism through the hexosamine biosynthetic pathway produces UDP-β-d-N-acetylglucosamine, an active sugar donor for O-linked β-N-acetylglucosamine modification (O-GlcNAcylation). (Nagel et al, 2013). O-GlcNAcylation is the glycosylation process through which N-acetylglucosamine (O-GlcNAc) gets added to serine and threonine residues of proteins. O-GlcNAcylation has been shown to stimulate chondrogenesis and osteogenesis and correlates with the transcriptional activity of the osteogenesis regulator, Runx2.

Heath and colleague (Heath et al, 2014) identified O-GlcNAcylation of AKT on T430 and T479 amino acids as a potential regulator of diabetes mellitus–induced calcification. In streptozotocin-treated mice, they found a strong increase in vascular O-GlcNAcylation along with increases in vascular calcification. Blocking the removal of O-GlcNAc further enhanced calcium levels both in vitro in cultured VSMCs and in vivo in mice.

Shao and colleague (Shao et al, 2005) studied endothelial cell dysfunction in diabetic patients. Major risk factors for endothelial cell dysfunction in T1D are poor glycemic control and diabetes mellitus duration, whereas in T2DM, insulin resistance is an important risk factor. The mechanisms by which hyperglycemia induces the activation of various pathways (DAG, PKC, and hexosamine) of glucose metabolism and production of oxidative stress, formation of AGEs, etc, also apply to endothelial cells. Driven by hyperglycemia and oxidative stress, apoptosis of endothelial cells and their overall dysfunction may promote endothelial permeability, exposing VSMCs to hyperglycemia and other proinflammatory circulating factors known to promote calcification such as ALP and receptor activator of nuclear factor-κB ligand. Moreover, production of tumor necrosis factor-α from both endothelial and SMCs induces production of BMP-2, a potent osteoblastic differentiation factor, which promotes osteogenenesis by activating the homeobox homolog (Msx2) and Wnt signaling pathways.

Milica and colleague (Milica et al, 2017) examined coronary artery calcium score (CACS) in 70 subjects with prediabetes. CAC score of 0 was present in 32 subjects. Minimal calcifications with a CAC score of 1–10 AU were present in 12 subjects with pre DM. moderate calcification of11–100 AU were present in 15 subjects. Nine subjects had significant calcifications with 101–400 AU. Two subjects had a CAC score of 100 AU which meant significant calcifications. Score risk below 2% was present in only 19 subjects, while a score risk of 15% and more was present in 23 subjects. No significant correlation was found between Score charts and CAC. However, a trend of finding more calcifications in those with a 10% and above Score risk was noted. In conclusion, an approach to risk assessment that combines the traditional risk factor-based paradigm with a more personalized atherosclerosis-imaging model may be appropriate for high-risk individuals, such as subjects with prediabetes.

In conclusion, diabetes and prediates associated with glucose metabolism, hexosamine biosynthetic pathway, UDP-β-d-N-acetylglucosamine, and O-linked β-N-acetylglucosamine modification (O-GlcNAcylation). O-GlcNAcylation stimulates vascular chondrogenesis, osteogenesis, and calcifications.

4. Framingham Risk Score (FRS) and CAC

The Framingham Risk Score (FRS) is an older popular tool widely used in clinical practice and research studies. It calculates the risk of CAD events (angina, myocardial infarction, and coronary death) over a 10 years period in asymptomatic patients. Risk factors used in Framingham scoring include age, sex, total cholesterol, HDL cholesterol, smoking, and blood pressure. The score has been validated in many populations, such as Caucasian Americans and African- Americans. This score was implemented in several guidelines for CVD prevention and performed CACS, seen on figure 5.2, and has been used to guide treatment of risk factors (Berger et al., 2010; D'Agostino et al., 2001).

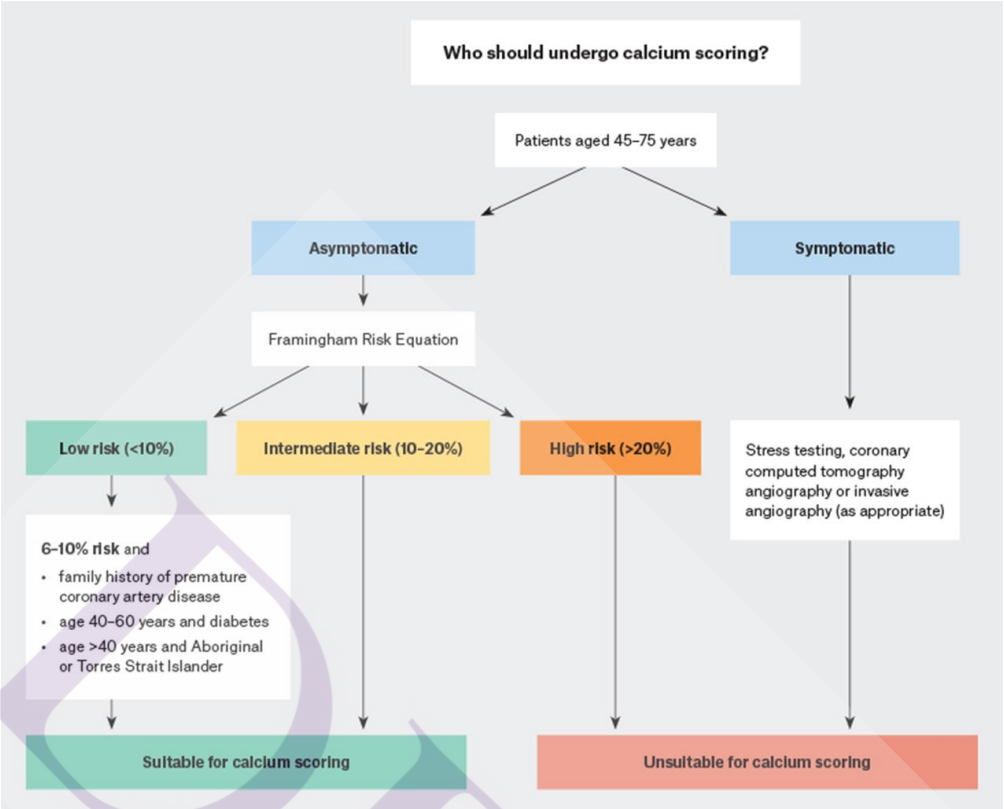


Figure 5.2: Diagram CACS guideline

Source: ACC/AHA guideline, 2016

The atherosclerotic cardiovascular disease risk calculator (ASCVD) is defined as nonfatal myocardial infarction, coronary heart disease, or stroke. The Pooled Cohort Equation (PCE) estimates the 10-year primary risk of ASCVD event developed by the American College of Cardiology /American Health Association. It was validated among Caucasian and African American and used only for adult patients without clinical ASCVD in age between 40-79 years (Goff et al., 2014). The risk factors used in this model were age, gender, race, total cholesterol, HDL cholesterol, SBP, treatment of BP, diabetes mellitus and smoking. Patients are considered to be at elevated risk if the pooled cohort equation predicts a risk of >7.5%, in this condition the 2013 ACC/AHA guidelines recommended the use of statin (Stone et al., 2013).

The 2018 ACC/AHA Cholesterol Guideline suggests that coronary artery calcium (CAC) testing may be considered in adults 40-75 years of age without diabetes mellitus and with LDL-C levels ≥ 70 mg/dl-189 mg/dl at a 10-year atherosclerotic cardiovascular disease (ASCVD) risk of ≥7.5% to <20% (i.e., intermediate risk group) if a decision about statin therapy is uncertain. In such patients, if CAC is zero, treatment with statin therapy may be withheld or delayed, except in cigarette smokers, those with diabetes mellitus, and those with a strong family history of premature ASCVD. According to the guideline, a CAC score of 1 to 99 favors statin therapy, especially in those ≥55 years of age. For any patient, if the CAC score is ≥100 Agatston units or ≥75th percentile, statin therapy is indicated unless otherwise deferred by the outcome of clinician-patient risk discussion.

In conclusion, CACS screening is of suitable for asymptomatic patient with low to intermediate cardiovascular risk (FRS <20%). In contrast, symptomatic patient should undergo CT angiography (CTA) of coronary artery or cardiac catheterization for diagnosis and treatment of coronary artery stenosis or thrombosis.

5. Dyslipidemia and positive CAC

Dyslipidemia is defined by the elevation or attenuation of serum lipids. Cholesterol and triglycerides are the major lipoproteins. To date, there is no evidence that fasting is superior to non-fasting in evaluating a lipid profile for cardiovascular risk prediction, Many countries are currently in the process of modifying their guidelines for measuring a lipid profile in the nonfasting state, which facilitates blood collection for patients, laboratory technicians and clinician (Langsted & Nordestgaard, 2019; Nordestgaard & Varbo, 2014).

All lipoproteins have a common basic structure but they differ in their size, density composition and chemical proprieties (Yusuf et al., 2004). The different lipoproteins are including chylomicrons, Intermediate Density Lipoprotein (IDL), Very Low-Density Lipoprotein (VLDL), LDL, HDL, and apolipoproteins such as (Apo A, apo B, apo C and apo E). Lipids disorders are

defined as the total cholesterol, Low density Lipoprotein Cholesterol (LDL-C), High density Lipoprotein-Cholesterol (HDL-C) and triglycerides. According to the large epidemiological studies, the results of a meta-analysis including 10 large cohort studies reported that for each 0.6 mmol/l or 23 mg/dl reduction in serum cholesterol levels in subjects > 60 years old, decrease the risk of CHD by 27% (Law, Wald & Thompson, 1994).

The National Cholesterol Education Program Adult Treatment Panel III of (NCEP ATPIII) define dyslipidemia as total cholesterol ≥ 240 mg/dl, triglycerides > 200 mg/dl, (LDL-C) >160 mg/dl and (HDL -C) < 50 mg/dl in women (N.C.E.P, 2002). The National Health and Nutrition Examination Survey (NHANES) reported a prevalence of total cholesterol level ≥ 240mg/dl, in USA population up to 33.6% (Tóth, Potter & Ming, 2012). In Middle east the prevalence of dyslipidemia was 70.5% (Labarthe, 2010; Yusuf et al., 2004). A study among adult population in GCC found that the prevalence of hypercholesteremia defined as total cholesterol >200 mg/dl ranged from 17% to 54.9% in males and 9% to 53.2% in females (Aljefree & Ahmed, 2015). A meta-analysis including 90,056 subjects in 14 randomized trial of statin showed that lowering LDL-C by 39 mg /dl was associated with one-fifth reduction in the 5 years incidence of major cardiovascular events (CAD, and stroke) (Baigent et al., 2005). The negative association between low HDL-C and the risk of heart disease is well defined. In Prospective Cardiovascular Munster (PROCAM) study, subjects with HDL-C < 35 mg/dl have 4-fold higher cardio vascular risk (Assmann et al., 1996). The Israeli Ischemic Heart Disease Study showed that subgroup with low HDL-C concentration had 36% greater CVD mortality than subgroups with elevated HDL-C (even after adjusted for age and CVRF) (Goldbourt, Yaari & Medalie, 1997). In addition, a meta-analysis of four studies demonstrated that for every 1mg /dl increase in HDL-C level there was decrease in coronary events risk by 2-3% independently of LDL-C (Gordon et al., 1989).

Triglycerides measurement is important for evaluating the risk of CVD mainly in diabetics, glucose intolerance, and insulin resistance. In the Copenhagen City Heart Study and the Women's Health Study, the increase in non-fasting triglycerides concentration by 5mmol/l versus less than

1mmol/l, was strongly associated with increasing adjusted age risks by 17- fold for MI, by 6 for IHD, 5 for ischemic stroke, and 4 for all-cause mortality in women.

For men the corresponding risks increases were by 5, 3, and 2-fold (Freiberg et al., 2008; Nordestgaard et al., 2007). Non-HDL cholesterol (Non-HDL-C) is the sum of cholesterol collected in all lipoprotein except HDL-C. It is calculated as the difference between total and HDL-C. It should be higher by about 30mg /dl than LDL-C. An elevation of non-HDL-C by 1mg/dl increases the risk of death due to CVD by 5%. It is also considered the second goal after LDL-C in diabetics patients (Bergmann, 2010)

The prevalence of overweight in men and women differs according to the level of the development of the country. Higher BMI is more prevalent in men than women in HIC, conversely in LMIC mainly in Arab countries a female predominance was described (Anon, 2015). Total cholesterol confers the same risk of cardiovascular in both sexes.

However, LDL-C increases cardiovascular risk in men more than women (Manolio et al., 1992). Low HDL-C represents equal risk for CAD in both sexes, mainly young age, but predicts CAD mortality in women more than in men. In addition, triglycerides are a part of metabolic syndrome which is higher in women. Smoking increases risk of cardiovascular events by 3.6 in women and 2.4 in men (Willett et al., 1987). A meta-analysis from 74 prospective cohort studies showed that women who smoke had a 25% greater relative risk of CAD than men (Huxley & Woodward, 2011).

In conclusion, increasing of weight, BMI, total cholesterol, LDL-C, non-HDL-C, and triglyceride are strongly associated with cardiovascular disease (MI, CAD, and IHD).

6. Serum uric acid and cardiovascular disease

Excessive intake of fructose, primarily in the form of added dietary sugars, has also been linked epidemiologically with the development of obesity, diabetes, and nonalcoholic fatty liver

disease. More importantly, the administration of fructose to humans induces all of the features of metabolic syndrome.

In a study by Stanhope *et al.* (2009), a diet containing 25% fructose was administered for 6 weeks to overweight adults; control subjects received 25% glucose. Subjects on the high-fructose diet developed insulin resistance, visceral obesity (measured by computed tomography scan), and postprandial dyslipidemia. In the Menorca study, the administration of 200 g fructose per day resulted in a 25% increase in metabolic syndrome, with a significant increase in fasting triglycerides, a fall in HDL cholesterol, a rise in systolic and diastolic BP, and a worsening of insulin resistance measured using the homeostasis model assessment index.

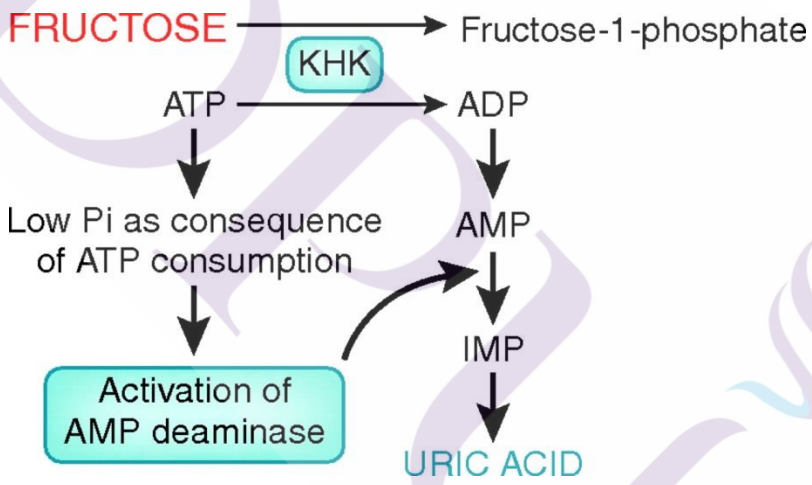


Figure 5.3: A pathway by which fructose is metabolized into uric acid. KHK, ketohexokinase.

Source: Richard J. Johnson et al (2010)

Uric acid (UA) is a potent endogenous antioxidant (Cristina, 2016). However, high concentrations of this molecule have been associated with cardiovascular disease (CVD) and renal dysfunction, involving mechanisms that include oxidative stress, inflammatory processes, and endothelial injury. Experimental and in vitro results suggest that this biomarker behaves like other antioxidants, which can shift from the physiological antioxidant action to a pro-oxidizing effect

according to their level and to microenvironment conditions. However, data on patients (general population or CAD cohorts) are controversial, so the debate on the role of hyperuricemia as a causative factor for CVD is still ongoing. Increasing evidence indicates UA as more meaningful to assess CVD in women, even though this aspect needs deeper investigation. It will be important to identify thresholds responsible for UA "biological shift" from protective to harmful effects in different pathological conditions, and according to possible gender-related differences. In any case, UA is a low-tech and inexpensive biomarker, generally performed at patient's hospitalization and, therefore, easily accessible information for clinicians. For these reasons, UA might represent a useful additive tool as much as a CV risk marker. Thus, in view of available evidence, progressive UA elevation with levels higher than 6 mg/dl could be considered an "alarm" for increased CV risk.

Presently, whether UA represents an independent risk factor for cardiovascular events with a direct and causal role or if it is just a marker for an adverse risk profile is still conjectural. UA is known as a potent antioxidant, proposed as an evolutionary alternative to the loss of ability in synthesizing ascorbate in higher primates, thus lowering the lipid peroxidation rate and counteracting the increased oxidative stress status. Accordingly, UA's protective role has been evidenced in several neurologic diseases, including multiple sclerosis and Parkinson's disease. These facts may argue in favor of a compensatory mechanism. Nonetheless, elevated UA might induce CV and renal disease, involving mechanisms characterized by oxidative stress, inflammation, and endothelial dysfunction. Specifically, UA can act as a pro-oxidant by generating free radicals during its degradation through xanthine oxidase activity, seen on Figure 5.4

Moreover, UA results correlated with different inflammatory parameters in a general population of the In CHIANTI study and in CAD patients, although it is not clear whether UA could represent a marker of pro-inflammatory state rather than a cause of inflammation. In this context, in vitro data suggest that UA induces monocyte apoptosis through the activation of both death receptor and mitochondrial-mediated pathways, and stimulates mononuclear cells to produce

TNFα. Moreover, recent data evidenced a role for UA in NLRP3 inflammation activation, which is critical for the release of inflammatory cytokines.

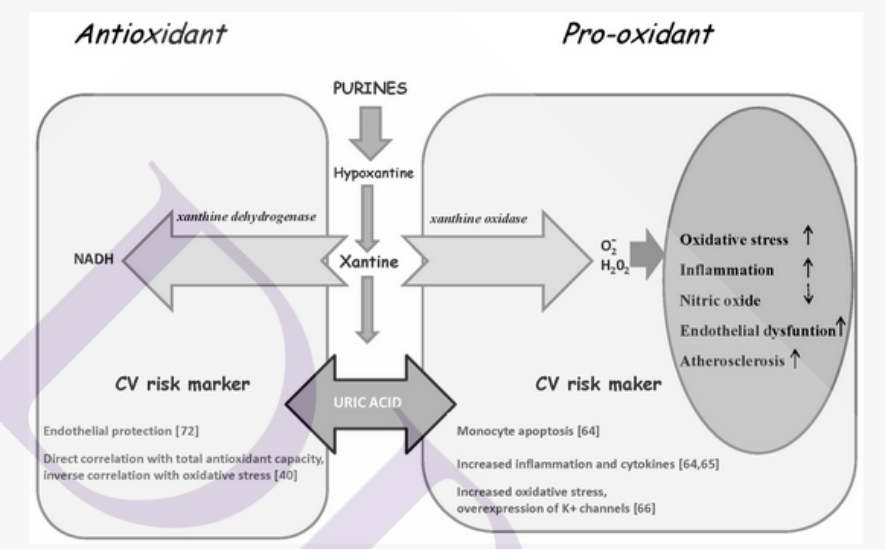


Figure 5.4: Mechanism of uric acid associated with monocyte apoptosis, increased inflammation and cytokines, increased endothelial dysfunction, and increased atherosclerosis

Source: Cristina et al (2016)

Table 5.1: Hyperuricemia risk factor for CVD results from meta-analysis studies

Table 1. Hyperuricemia as risk factor for CVD: results from meta-analysis studies.

Patient Number	Subjects	Study Number	Endpoint	Odds Ratio (95%CI)	Comment	Ref.
402.997	general population	13	Coronary heart disease, CHD	1.09 (1.03–1.16)	Higher risk for CHD mortality in women	[44]
		9	CHD mortality	1.16 (1.01–1.30)		
			CHD mortality	1.12 (1.05–1.19)		
9.458	CHD patients	16	CHD	1.13 (1.07–1.20)	1.02 (CI, 0.91–1.14) in 8 studies with more complete adjustment	[45]
155.084	controls					
172.123	general population	11	Cardiovascular mortality	1.37 (1.19–1.57)	Higher risk for CV mortality in women	[46]
			all-cause mortality	1.24 (1.09–1.42)	Higher risk for all-cause mortality in men	
457.915	general population	12	CHD incidence	1.21 (1.07–1.36)	Higher risk for CHD incidence and mortality in women	[47]
237.433		7	CHD mortality	1.21 (1.00–1.46)		
12.677	complicated myocardial infarction (MI) or	3	CV mortality	1.47 (1.17–1.83)		[80]
	heart failure (HF)		all-cause mortality	1.36 (1.11–1.67)		
			HF hospitalization	1.28 (1.14–1.43)		
427.917	general population and CHD pts	5	HF incidence	1.19 (1.17–1.21)		[81]
51.552	HF patients	28	all-cause mortality	1.04 (1.02–1.06)		
			death or cardiac events	1.28 (0.97–1.70)		

Source: Cristina et al (2016)

In conclusion, the recent available data about association of hyperuricemia and cardiovascular risk factors are not definitive, seen on table 5.1. However, routine administration of 200g fructose per day resulted in a 25% increase in metabolic syndrome, with a significant increase in fasting triglycerides, a fall in HDL, a rise in systolic and diastolic BP, and increased insulin resistance. A more critical aspect will be to define the threshold responsible for UA "biological shift" from protective to dangerous actions and the possible role of elevated UA in the interaction with other toxic substrates and reactive oxygen species in order to contribute to vessel damage and dysfunction.

Suggestion

According to an objective study and the results of correlation between prediabetic status (HbA1c 5.7 to 6.4%), cardiovascular risks (determined by Framingham Risk Score: FRS) and

coronary artery calcium (CAC), by retrospective analysis of 5 years data documents, Jan 2015 to Dec 2020., the suggestions are follows;

1. Prediabetic stage (HbA1c 5.7 to 6.4%) should be concern in clinical practice about coronary artery calcification and cardiovascular risk.
2. Combination cardiovascular risk assessment by Framingham Risk Score (FRS) and CT coronary artery calcium score (CACS) should be performed in prediabetic cases
3. CACS can be used as bioimaging atherosclerotic marker in prediabetic case with cardiovascular risk factor.
4. Prediabetes with low to intermediate cardiovascular risk (FRS <20%) with CACS 1 to 100 must be received the strong recommendation of life style modification for optimal health and prevent further cardiovascular event.

Bibliography

- Adeney KL, Siscovick DS, Ix JH, Seliger SL, Shlipak MG, Jenny NS, Kestenbaum BR. Association of serum phosphate with vascular and valvular calcification in moderate CKD.**J Am Soc Nephrol**. 2009; 20:381–387.
- Arad Y, Goodman KJ, Roth M, Newstein D, Guerci AD. Coronary calcification, coronary disease risk factors, C-reactive protein, and atherosclerotic cardiovascular disease events: the St. Francis Heart Study. **J Am Coll Cardiol**. 2005; 46:158–165
- Arenja N, Mueller C, Ehl NF, Brinkert M, Roost K, Reichlin T, Sou SM, Hochgruber T, Osswald S, Zellweger MJ. Prevalence, extent, and independent predictors of silent myocardial infarction. **Am J Med**. 2013; 126:515–522.
- Aleopoulos N, Raggi P. Calcification in atherosclerosis. **Nat Rev Cardiol**. 2009; 6:681–688. doi: 10.1038/nrcardio.2009.165.CrossrefMedlineGoogle Scholar
- Amann K. Media calcification and intima calcification are distinct entities in chronic kidney disease. **Clin J Am Soc Nephrol**. 2008; 3:1599–1605.
- American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In Standards of Medical Care in Diabetes 2017. *Diabetes Care* 2017; 40(Suppl. 1): S11– 24.
- American College of Cardiology (ACC) and American Heart Association (AHA), ACC/AHA guideline. 2018
- Blaha MJ, Cainzos-Achirica M, Greenland P, McEvoy JW, Blankstein R, Budoff MJ, Dardari Z, Sibley CT, Burke GL, Kronmal RA, Szklo M, Blumenthal RS, Nasir K. Role of coronary artery calcium score of zero and othernegative risk markers for cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis (MESA). **Circulation**. 2016; 133:849–858.
- Block GA, Port FK. Re-evaluation of risks associated with hyperphosphatemia and hyperparathyroidism in dialysis patients: recommendations for a change in management. **Am J Kidney Dis**. 2000; 35:1226–1237.

- Budoff MJ, Dowe D, Jollis JG, Gitter M, Sutherland J, Halamert E, Scherer M, Bellinger R, Martin A, Benton R, Delago A, Min JK. Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial. **J Am Coll Cardiol**. 2008; 52:1724–1732.
- Burke AP, Kolodgie FD, Farb A, Weber DK, Malcom GT, Smialek J, Virmani R. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. **Circulation**. 2001; 103:934–940.
- Burke AP, Kolodgie FD, Farb A, Weber D, Virmani R. Morphological predictors of arterial remodeling in coronary atherosclerosis. **Circulation**. 2002; 105:297–303.
- Burke AP, Kolodgie FD, Zieske A, Fowler DR, Weber DK, Varghese PJ, Farb A, Virmani R. Morphologic findings of coronary atherosclerotic plaques in diabetics: a postmortem study. **Arterioscler Thromb Vasc Biol**. 2004; 24:1266–1271. doi: 10.1161/01.ATV.0000131783.74034.97.
- Burke AP, Kolodgie FD, Farb A, Virmani R. Pathogenesis and significance of calcification in coronary atherosclerosis., Virmani R, Narula J, Leon MB, Willerson JT, eds. **In: The Vulnerable Atherosclerotic Plaque: Strategies for Diagnosis and Management**. 1st ed. Malden, MA: Wiley-Blackwell; 2007: 77–94.
- Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification.**Genes Dev**. 1998; 12:1260–1268.
- Byon CH, Sun Y, Chen J, Yuan K, Mao X, Heath JM, Anderson PG, Tintut Y, Demer LL, Wang D, Chen Y. Runx2-upregulated receptor activator of nuclear factor ΚB ligand in calcifying smooth muscle cells promotes migration and osteoclastic differentiation of macrophages. **Arterioscler Thromb Vasc Biol**. 2011; 31:1387–1396

- Carson AP, Steffes MW, Carr JJ, Kim Y, Gross MD, Carnethon MR, Reis JP, Loria CM, Jacobs DR, Lewis CE. Hemoglobin a1c and the progression of coronary artery calcification among adults without diabetes. **Diabetes Care**. 2015; 38:66–71.
- Chang Y, Yun KE, Jung HS, Kim CW, Kwon MJ, Sung E, Ryu S. A1C and coronary artery calcification in nondiabetic men and women. **Arterioscler Thromb Vasc Biol**. 2013; 33:2026–2031.
- Cheng VY, Lepor NE, Madyoon H, Eshaghian S, Naraghi AL, Shah PK. Presence and severity of noncalcified coronary plaque on 64-slice computed tomographic coronary angiography in patients with zero and low coronary artery calcium. **Am J Cardiol**. 2007; 99:1183–1186.
- Cristina Vasselle, Annamaria Mazzone, Laura Sabatina and Clara Carpeggini. Uric Acid for Cardiovascular Risk: Dr Jekyll or Mr.Hide?.**Disease J**.2016; 4(1):1-11.
- Davies MJ. The composition of coronary-artery plaques. **N Engl J Med**. 1997; 336:1312–1314.
- Demer LL, Tintut Y. Inflammatory, metabolic, and genetic mechanisms of vascular calcification. **Arterioscler Thromb Vasc Biol**. 2014; 34:715–723.
- Esposito L, Saam T, Heider P, Bockelbrink A, Pelisek J, Sepp D, Feurer R, Winkler C, Liebig T, Holzer K, Pauly O, Sadikovic S, Hemmer B, Poppert H. MRI plaque imaging reveals high-risk carotid plaques especially in diabetic patients irrespective of the degree of stenosis. **BMC Med Imaging**. 2010; 10:27-34.
- Evrard S, Delanaye P, Kamel S, Cristol JP, Cavalier E; SFBC/SN Joined Working Group on Vascular Calcifications. Vascular calcification: from pathophysiology to biomarkers. **Clin Chim Acta**. 2015; 438:401–414.
- Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J, Virmani R. Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. **Circulation**. 1996; 93:1354–1363.

- Fitzpatrick LA, Severson A, Edwards WD, Ingram RT. (1994). Diffuse calcification in human coronary arteries: association of osteopontin with atherosclerosis. **J Clin Invest.;** 94:1597-1604.
- Friedlander AH, Maeder LA. The prevalence of calcified carotid artery atheromas on the panoramic radiographs of patients with type 2 diabetes mellitus. **Oral Surg Oral Med Oral Pathol Oral Radiol Endod**. 2000; 89:420–424.
- Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. **N Engl J Med**. 1987; 316:1371–1375.
- Giachelli CM, Bae N, Almeida M, Denhardt DT, Alpers CE, Schwartz SM. Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. **J Clin Invest**. 1993; 92:1686–1696.
- Gyongyosi M, Yang P, Hassan A, Weidinger F, Domanovits H, Laggner A, Glogar D. Coronary risk factors influence plaque morphology in patients with unstable angina. **Coron Artery Dis**. 1999; 10:211–219.
- Harper E, Forde H, Davenport C, Rochfort KD, Smith D, Cummins PM. Vascular calcification in type-2 diabetes and cardiovascular disease: Integrative roles for OPG, RANKL and TRAIL. **Vascul Pharmacol**. 2016; 82:30–40.
- Heath JM, Sun Y, Yuan K, Bradley WE, Litovsky S, Dell'Italia LJ, Chatham JC, Wu H, Chen Y. Activation of AKT by O-linked N-acetylglucosamine induces vascular calcification in diabetes mellitus. **Circ Res**. 2014; 114:1094–1102
- Hofmann Bowman MA, Gawdzik J, Bukhari U, Husain AN, Toth PT, Kim G, Earley J, McNally EM. S100A12 in vascular smooth muscle accelerates vascular calcification in apolipoprotein E-null mice by activating an osteogenic gene regulatory program. **Arterioscler Thromb Vasc Biol**. 2011; 31:337–344.
- Ho CY, Shanahan CM. Medial arterial calcification: an overlooked player in peripheral arterial disease.**Arterioscler Thromb Vasc Biol**. 2016; 36:1475–1482.

- Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. **N Engl J Med**. 2008; 359:1577–1589
- International Diabetes Federation. IDF Diabetes Atlas, 8th edn. Brussels, Belgium: International Diabetes Federation, 2017
- Jung CH, Rhee EJ, Kim KJ, Kim BY, Park SE, Chang Y, Ryu S, Park CY, Mok JO, Oh KW, Kim CH, Park SW, Kang SK, Lee WY. Relationship of glycated hemoglobin A1c, coronary artery calcification and insulin resistance in males without diabetes. **Arch Med Res**. 2015; 46:71–77.
- Kannel WB, Vasan RS. Is age really a non-modifiable cardiovascular risk factor? **Am J Cardiol**. 2009 Nov 1;104(9).
- Kay AM, Simpson CL, Stewart JA. The role of AGE/RAGE signaling in diabetes-mediated vascular calcification. **J Diabetes Res**. 2016; 2016:609-703.
- Kim HS, Chung W, Kim AJ, Ro H, Chang JH, Lee HH, Jung JY. Circulating levels of soluble receptor for advanced glycation end product are inversely associated with vascular calcification in patients on haemodialysis independent of S100A12 (EN-RAGE) levels. **Nephrology (Carlton)**. 2013; 18:777–782.
- Khazai B, Luo Y, Rosenberg S, Wingrove J, Budoff MJ. Coronary Atherosclerotic plaque detected by computed tomographic angiography in subjects with diabetes compared to those without diabetes. **PLoS One**. 2015; *10;* 1-11
- Lam DW, LeRoith D. The worldwide diabetes epidemic. **Curr Opin Endocrinol Diabetes Obes**. 2012; 19:93–96.
- Lanzer P, Boehm M, Sorribas V, Thiriet M, Janzen J, Zeller T, St Hilaire C, Shanahan C. Medial vascular calcification revisited: review and perspectives. **Eur Heart J**. 2014; 35:1515–1525.
- Levin L, Tomer Y. The etiology of autoimmune diabetes and thyroiditis: evidence for common genetic susceptibility. **Autoimmun Rev**. 2003; 2:377–86.

- Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. **Arch Intern Med**. 2004; 164:2147–2155.
- Mauriello A, Sangiorgi G, Palmieri G, Virmani R, Holmes DR, Schwartz RS, Pistolese R, Ippoliti A, Spagnoli LG. Hyperfibrinogenemia is associated with specific histocytological composition and complications of atherosclerotic carotid plaques in patients affected by transient ischemic attacks. **Circulation**. 2000; 101:744–750.
- Malmberg K, Yusuf S, Gerstein HC, Brown J, Zhao F, Hunt D, Piegas L, Calvin J, Keltai M, Budaj A. Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry. **Circulation**. 2000; 102:1014–1019.
- Milica Marjanovic Petkovic, Ivana Sasic, Miljanka Vuksanovic & Teodora Beljic Zivkovic. Coronary artery calcium score in prediabetes - preliminary results. **Endrocrine** *Abstracts*.2017; *86:* 49 -54.
- Moreno PR, Murcia AM, Palacios IF, Leon MN, Bernardi VH, Fuster V, Fallon JT. Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus. **Circulation**. 2000; 102:2180–2184.
- Mukamal KJ, Nesto RW, Cohen MC, Muller JE, Maclure M, Sherwood JB, Mittleman MA. Impact of diabetes on long-term survival after acute myocardial infarction: comparability of risk with prior myocardial infarction. **Diabetes Care**. 2001; 24:1422–1427.
- Nadra I, Mason JC, Philippidis P, Florey O, Smythe CD, McCarthy GM, Landis RC, Haskard DO. Proinflammatory activation of macrophages by basic calcium phosphate crystals via protein kinase C and MAP kinase pathways: a vicious cycle of inflammation and arterial calcification? **Circ Res**. 2005; 96:1248–1256.
- Nagel AK, Schilling M, Comte-Walters S, Berkaw MN, Ball LE. Identification of O-linked N-acetylglucosamine (O-GlcNAc)-modified osteoblast proteins by electron transfer

dissociation tandem mass spectrometry reveals proteins critical for bone formation. **Mol Cell Proteomics**. 2013; 12:945–955.

- Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. **Nature**. 2000; 404:787–790.
- Nour Tashtish, Sadeer Al-Kindi, Anuja Sarode, Jared Derieux, Robert Gilkeson, Sanjay Rajagopalan. Coronary artery calcium score and cardiovascular events in patients with diabetes, prediabetes, and no Diabetes**. Circulation**. 2019; *11*:140-148.
- North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. **Circ Res.** 2012 Apr 13;110(8):1097-108.
- Odink AE, van der Lugt A, Hofman A, Hunink MG, Breteler MM, Krestin GP, Witteman JC. Risk factors for coronary, aortic arch and carotid calcification; The Rotterdam Study.**J Hum Hypertens**. 2010; 24:86–92.
- Olson JC, Edmundowicz D, Becker DJ, Kuller LH, Orchard TJ. Coronary calcium in adults with type 1 diabetes: a stronger correlate of clinical coronary artery disease in men than in women. **Diabetes**. 2000; 49:1571–1578.
- Otsuka F, Sakakura K, Yahagi K, Joner M, Virmani R. Has our understanding of calcification in human coronary atherosclerosis progressed?**Arterioscler Thromb Vasc Biol**. 2014; 34:724–736.
- Panizo S, Cardus A, Encinas M, Parisi E, Valcheva P, López-Ongil S, Coll B, Fernandez E, Valdivielso JM. RANKL increases vascular smooth muscle cell calcification through a RANK-BMP4-dependent pathway. **Circ Res**. 2009; 104:1041–1048
- Raggi P, Shaw LJ, Berman DS, Callister TQ. Prognostic value of coronary artery calcium screening in subjects with and without diabetes. **J Am Coll Cardiol**. 2004; 43:1663–1669.
- Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, Jahnen-Dechent W, Weissberg PL, Shanahan CM. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and

phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD.**J Am Soc Nephrol**. 2004; 15:2857–2867.

- Richard J, Gabreita S, and Takahiko N, The effect of fructose on renal biology and disease. J Am Society of Nephrology. 2010; 21(12):2036-2039
- Rossi R., Grimaldi T., Origliani G., Coppi F., Modena M.G. Menopause and cardiovascular risk. **Journal of Pathophysiology of Haemostasis and Thrombosis**. 2002;32:325-328.
- Scicali R, Giral P, Antonino A, MariaRabuazzo A, Purrello F, Cluzel P, Redheuil A, Bruckert E, Rosenbaum D. HbA1c increase is associated with higher coronary and peripheral atherosclerotic burden in non diabetic patients. **Atherosclerosis.** 2016;255: 102-108
- Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. **Circulation**. 1995; 92:2157–2162.
- Sarwar N, Gao P, Seshasai SR. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. **Lancet**. 2010; 375:2215–22.
- Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. **Diabetes Care**. 2009; 32:525–527.
- Shang Y, Marseglia A., Fratiglioni L., Welmer A.-K., Wang R, Wang H.-X., and Xu W. Natural history of prediabetes in older adults from a population-based longitudinal study. **Journal of Internal Medicine**. 2019; 286:326-340.
- Shemesh J, Tenenbaum A, Fisman EZ, Koren-Morag N, Grossman E. Coronary calcium in patients with and without diabetes: first manifestation of acute or chronic coronary events is characterized by different calcification patterns. **Cardiovasc Diabetol**. 2013; 12:161.

- Selvin E, Coresh J, Golden SH, Brancati FL, Folsom AR, Steffes MW. Glycemic control and coronary heart disease risk in persons with and without diabetes: the atherosclerosis risk in communities study. **Arch Intern Med**. 2005; 165:1910–1916.
- Speer MY, Yang HY, Brabb T, Leaf E, Look A, Lin WL, Frutkin A, Dichek D, Giachelli CM. Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. **Circ Res**. 2009; 104:733–741.
- Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, McGahan JP, Seibert A, Krauss RM, Chiu S, Schaefer EJ, Ai M, Otokozawa S, Nakajima K, Nakano T, Beysen C, Hellerstein MK, Berglund L, Havel PJ.: Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. **J Clin Invest**. 2009; 119: 1322–1334.
- Perez-Pozo SE, Schold J, Nakagawa T, Sanchez-Lozada LG, Johnson RJ, Lillo J.: Excessive fructose intake induces the features of metabolic syndrome in healthy adult men: Role of uric acid in the hypertensive response. **Int J Obes** (Lond).2010; 34: 454–461.
- Tanenbaum SR, Kondos GT, Veselik KE, Prendergast MR, Brundage BH, Chomka EV. Detection of calcific deposits in coronary arteries by ultrafast computed tomography and correlation with angiography. **Am J Cardiol**. 1989; 63:870–872.
- Virmani R, Burke AP, Kolodgie F. Morphological characteristics of coronary atherosclerosis in diabetes mellitus. **Can J Cardiol**. 2006; 22(suppl B):81B–84B.
- Weissman NJ, Sheris SJ, Chari R, Mendelsohn FO, Anderson WD, Breall JA, Tanguay JF, Diver DJ. Intravascular ultrasonic analysis of plaque characteristics associated with coronary artery remodeling. **Am J Cardiol**. 1999; 84:37–40.
- Yahagi K, Davis HR, Arbustini E, Virmani R. Sex differences in coronary artery disease: pathological observations. **Atherosclerosis**. 2015; 239:260–267.
- Zavodni AE, Wasserman BA, McClelland RL, Gomes AS, Folsom AR, Polak JF, Lima JA, Bluemke DA. Carotid artery plaque morphology and composition in relation to

incident cardiovascular events: the Multi-Ethnic Study of Atherosclerosis (MESA).**Radiology**. 2014; 271:381–389.

- Zhang M, Sara JD, Wang FL, Liu LP, Su LX, Zhe J, Wu X, Liu JH. Increased plasma BMP-2 levels are associated with atherosclerosis burden and coronary calcification in type 2 diabetic patients.**Cardiovasc Diabetol**. 2015; 14:64.

Biography of Researcher

Name	Thunnawat Wattanaseth
Date of birth	May 25, 1977
Nationality	Thai
Education	2001: Doctor of Medicine (M.D.), Naresuan University, Thailand 2005: Diploma of Diagnostic Radiology, Chulalongkorn University Hospital, Bangkok, Thailand. 2007: Certificate of Comprehensive CTA-Coronary Artery level 4 of 4 (Supervisor level), Lenox Hill Hospital. 2008: Research Fellowship in Diagnostic Neuroradiology, University of San Diego (UCSD), USA. 2009: Visiting Fellowship in functional MRI (fMRI), Harvard Medical School, Boston, USA. New York, USA. 2018: Doctor of Philosophy (Ph.D.) in Buddhist Psychology, MCU, Thailand. 2019: Master of Business Administration (M.B.A), Kirk University, Thailand. 2020: American Board of Anti-Anting and Regenerative Medicine (ABAARM), Chicago, Illinois, USA.
Published Research	Wattanaseth, T. (2018). A Study of Meditation Effect on The Brain and Emotional Happiness by MRI Scan. The Journal of International Association of Buddhist Universities (JIABU), 11(2), 178-199.
Present	(1) Director of MRI Center and Diagnostic Imaging Center, Kasemrad International Hospital., Nonthaburi, Thailand. (2) Instructor of Neuropsychology, Ph.D. Program of Buddhist Psychology, Faculty of Humanities, MCU, Thailand.