

## Challenges in Predicting Risks of Premature Coronary Artery Disease (PCAD) (Cabaran dalam Meramal Risiko Penyakit Arteri Koronari Pramatang (PCAD))

MUHAMMAD FAIZAN A. SHUKOR\*, NOOR AKMAL SHAREELA ISMAIL & WAN ZURINAH WAN NGAH

### ABSTRACT

*Coronary artery disease (CAD) predominantly manifests in older population above the age of 60 years old. The incidence of CAD in younger individuals has been reported and is called premature CAD (pCAD). The prevalence for pCAD in individuals below 45 years old is about 3-10% worldwide. Advances in risk prediction are of great importance as absolute values of risk factors sometimes correlate poorly with individuals. The measurement of traditional risk factors such as cholesterol level and blood pressure might be inadequate to predict risk for pCAD and therefore new biomarkers are required. The introduction of omics technology offers insight into the mechanism and interactions involved during disease progression and open the possibilities of discovering new biomarkers. Currently, new potential biomarkers for pCAD have been explored such as homocysteine, apolipoproteins, microRNAs and single nucleotide polymorphisms. In this review, we discussed the associated risk factors for pCAD, several reported and newly proposed biomarkers and their potential to be used clinically.*

*Keywords: Atherosclerosis; biomarker; premature coronary artery disease*

### ABSTRAK

*Penyakit arteri koronari (CAD) sering berlaku dalam populasi yang berumur 60 tahun ke atas. Walau bagaimanapun, kejadian dalam individu muda yang dipanggil CAD pramatang (pCAD) telah dilaporkan. Prevalens pCAD bagi individu di bawah umur 45 tahun adalah 3-10% di seluruh dunia. Kemajuan dalam meramal risiko adalah penting kerana nilai faktor risiko mutlak kadangkala berkorelasi lemah dengan individu. Pengukuran faktor risiko tradisi seperti paras kolesterol dan tekanan darah mungkin tidak mencukupi untuk meramal risiko pCAD, oleh itu penanda biologi baru diperlukan. Pengenalan teknologi omik memberikan lebih kefahaman terhadap mekanisme dan interaksi yang berlaku sewaktu perkembangan penyakit dan membuka kemungkinan untuk penemuan penanda biologi baru. Ketika ini, penanda biologi pCAD yang baru telah diteroka seperti homosisteina, apolipoprotein, mikroRNA dan polimorfisma nukleotida tunggal. Dalam ulasan ini, kami membincangkan tentang faktor risiko pCAD yang berkaitan, beberapa penanda biologi yang dilaporkan dan yang baru dicadangkan dan potensi mereka untuk digunakan secara klinikal.*

*Kata kunci: Aterosklerosis; penanda biologi; penyakit koronari arteri pramatang*

### INTRODUCTION

Coronary artery disease (CAD) remains one of the leading causes of death in the world, despite a decrease in mortality and morbidity rates in the past decade (Nichols et al. 2014). CAD is commonly found among older individuals, while the prevalence among the younger population varies between 3 and 10% (Mohan et al. 2001; Wilson et al. 1998). In America, the predisposition risk of cardiovascular disease in men between 30-34 years old is 3% and the risk increased to 21% when they reach 60-64 years old (Wilson et al. 1998). In Japan, the prevalence is 1.26 cases for every 1000 patients below 50 years old (Sato et al. 2006) while in India, the prevalence recorded is about 10% (Mohan et al. 2001). Another study reported that, only a small percentage (<10%) of all patients with myocardial infarction are among individuals below 45 years old (Morillas et al. 2007). In Malaysia, 24% of 16,872 ACS patients that have been admitted to 17 hospitals in Malaysia between 2006 and 2010 were

below 50 years old. 33% of them were active smokers, 33% were diagnosed with dyslipidemia, 61% were hypertension, 41% were diabetic and 11% have family history with premature CAD (pCAD) (Wan Ahmad & Sim 2013). Compared to other country databases, Malaysian who undergone percutaneous coronary intervention (PCI) were much younger and have higher prevalence for risk factors (Ahmad et al. 2013). Although most studies suggest a low incidence rate for pCAD, it is possible that the exact number of young individuals with atherosclerosis is probably much larger than currently estimated. In addition, the rise in cardiovascular risk factors such as smoking and obesity among younger population could lead to an increase in pCAD cases (Arzamendi et al. 2011). Therefore early detection and treatment of subclinical CAD and the prevention of developing acute coronary events should be emphasized among young adults.

Predicting risk and disease development is challenging and cardiovascular disease is no exception. Absolute values

of risk factors such as cholesterol level sometimes correlate poorly within individuals (Castelli 1996). Accurately determining the risk among the younger population is paramount if prevention strategies were to be targeted appropriately. Current understanding on pathobiology of atherosclerosis has linked inflammation (Libby 2012) and oxidative stress (Madamanchi et al. 2005) as the central contributors to the initiation and progression of coronary artery disease. Identifying associated markers that may have prognostic values for future cardiovascular events in high risk patients may be crucial. Importantly, it will be useful in predicting diseases among individuals with no apparent syndrome or in younger populations who tend to develop CAD earlier in life. This could improve early diagnosis and enhance prevention strategies. In this review, we discussed on the associated risk factors, biomarkers as well as novel approaches that has been proposed in the literature focusing on pCAD.

#### RISK FACTORS ASSOCIATED WITH PCAD

A number of variables has been suggested to be predictive of cardiovascular events and such variables qualify as risk factors for CAD. Risk factors can be categorized into two different groups - modifiable and non-modifiable. Modifiable risk factors such as smoking, cholesterol levels, blood pressure and diabetes mellitus can be controlled by lifestyle interventions while non-modifiable risk factors such as age, gender and family history are factors that cannot be altered (Table 1).

TABLE 1. Associated risk factors for CAD and pCAD

CAD risk factors	
Non-modifiable	Modifiable
Age	Smoking status
Sex	Blood pressure
Race	Diabetes Mellitus
Family history	Cholesterol levels
	Physical activity
	Obesity

#### GENDER

It is well-acknowledged that pCAD is highly associated with males. Several studies have reported differences in baseline characteristics and clinical presentations between men and women. For instance, women with acute coronary syndrome (ACS) in the Canadian population are significantly older and are more likely to have diabetes, hypertension and a history of heart failure (Poon et al. 2012). Studies from PROSPECT, AMIS PLUS Registry and Gulf RACE-2 have recorded similar findings (Lansky et al. 2012; Radovanovic et al. 2007; Shehab et al. 2013). It was also reported that women were more frequently to have normal or mild disease when presented to angiogram (Dey et al. 2009). Although the incidence of acute myocardial

infarction remains highest in men, women appeared to exhibit the worst prognosis (Milcent et al. 2007). Young women showed a greater post-infarction mortality in hospital and up to 1-year after discharge in comparison to men (4.23% vs. 2.21%,  $p = 0.005$ ) (Egiziano et al. 2013). A study on gender related risk factors showed that women have significantly higher frequency of diabetes, family history of CAD and hypertension. In addition, women with a positive family history showed the greatest risk factor burden including traditional risk factors of hypertension (67%), obesity (53%), dyslipidemia (67%), smoking (42%) and diabetes (33%) and non-traditional risk factors of depression (37%), anxiety (55%) and low household income (44%) (Choi et al. 2014). While women have higher rates of hypertension and diabetes, their background were seen less among the smokers (Shehab et al. 2013). Although the prevalence for smoking among women is lower, it has been shown to give a greater effect on women with 60 percent increased risk for coronary heart disease (CHD) as compared to men who smoke (Prescott et al. 1998).

#### FAMILY HISTORY WITH CAD

Family history of CAD has been established as an independent risk factor for CAD from multiple populations based studies. It is estimated that heredity contributes to at least 50% of susceptibility to CAD. Importantly, the strength of risk is higher with younger age of onset (Sesso et al. 2001). In a survey of 15,381 patients, the frequency of young patients having family history of CAD (43.6%) is significantly higher compared to late onset CAD (26.5%) (Reibis et al. 2012) supporting the contribution of heredity to the occurrence of pCAD. A subsequent study from CONFIRM Registry involving 6308 young patients has reported that those with a family history of CAD have higher prevalence of pCAD. After a follow-up period, patients with family history of CAD also experienced higher annual rates of myocardial infarction (Otaki et al. 2013). A similar observation is recorded in Framingham Offspring Study as parental cardiovascular disease has been found to independently predict future events in middle age offspring. Among 2302 subjects (mean age = 44 years), 164 men and 79 women had cardiovascular events during follow-up. Those who have at least one parent with pCAD had higher risk for events, with OR=2.6 (95% CI, 1.7-4.1) for men and 2.3 (95% CI, 1.3-4.3) for women after adjusted with age compared to control (Lloyd-Jones et al. 2004).

In asymptomatic first degree relative of patients with pCAD, the frequency of abnormal calcium scores is two-fold higher compared to control with OR=1.96 (95% CI 1.04 – 3.67,  $p < 0.05$ ) (Taraboanta et al. 2012). A non-premature parental coronary disease appears to be a weaker predictor (Lloyd-Jones et al. 2004). The risk frequency differs according to the population studied implying a genetic determinant or race-specific association for the disease. Multi ethnic comparison involving 13,591 patients from 3 ethnic groups (Malay, Chinese and Indian) in Malaysia

revealed that the Indians have the highest frequency of family history of pCAD suggesting a strong genetic predisposition to CAD in this ethnic group (Lu & Nordin 2013). White middle age group with any parental history of premature CVD (pCVD) also appear to have higher odds of coronary artery calcium than those with no parental history (OR= 1.55, 95% CI 1.01-2.37). Significant association has also been observed between parental pCVD with intima media thickness in Caucasians with paternal pCVD (OR= 1.93, 95% CI 1.10-3.39) or any parental pCVD (OR= 1.67, 95% CI 1.02-2.74) while no association has been observed in black participants (Wilkins et al. 2014).

#### SMOKING

Despite all efforts to reduce smoking on a global scale, smoking remains as one of the strongest risk factors for pCAD (Hbejan 2011; Hozawa et al. 2006). Previous research involving 200 patients below 35 years old who underwent coronary angiography reported that smoking (71%) is the most frequent risk factor in pCAD (Christus et al. 2011). Furthermore, the attributable proportions of CHD among women who smoke in 8 pooled cohort studies were highest in the young group with 88% for 40-49 years old, 81% for 50-59 years old, 71% for 60-69 years old and 68% for above 70 years old. The hazard ratio of CHD in current smokers relative to non-smokers also appears to be highest in the youngest and lowest in the oldest group in both men and women (Tolstrup et al. 2014). The relative risk for CAD for patients included in the Framingham Heart Study is about three times higher among young smokers as compared to the non-smokers. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) reported that smokers (32%) have over twice advanced lesions compared to the non-smokers (14%) (Zieske et al. 1999). Smokers also have greater prevalence of Grade 5 lesions compared to the non-smokers suggesting that smoking accelerates the transition from Grade 4 to Grade 5 lesions (Zieske et al. 2005). Although smoking cessation in young healthy smokers showed normalization of endothelial dysfunction, smoking effect is not reversible in middle-age adults (Naya et al. 2011).

#### CHRONIC DISEASES

Diabetes, hypertension and hypercholesterolemia are also frequently present in early and late onset of CAD. These are considered as modifiable risk factors but involve both environmental as well as genetic components. Whereas the significant involvement of these factors in CAD progression is well documented, their importance amongst pCAD is still poorly described. Most studies on angiography profiles of CAD patients showed that older patients are more diabetic (Pineda et al. 2008; Uddin et al. 2004). However, the frequency of cardiovascular risk factors such as overweight and high cholesterol among young people with type 1 diabetes mellitus remained common (Dobrovolskienė et al. 2013). Similarly in Malaysia, the prevalence for hypertension was higher in people above 60

years old (26.8%), while people between 40-49 years old have the highest prevalence for obesity (22.7%) (Yunus et al. 2004). Young hypertensive subjects with multiple risk factors including positive family history (OR= 12.317), low HDL (OR= 3.267) and hypertriglyceridemia (OR= 2.894) are significantly associated with pCAD (Che et al. 2013). Although hypercholesterolemia is a common risk in CAD, many researchers have reported a higher prevalence in younger group. The prevalence for this risk is about 73% in young patients compared to 59% in older patients (Pineda et al. 2008).

#### PATHOGENESIS OF CAD

The mechanism underlying the pathogenesis of CAD is the formation of atherosclerotic plaque characterized by deposition of cholesterol into the arterial wall. These events usually occur at the site of damaged endothelium possibly due to oxidative stress, physical forces or shear stress from the blood flow (Cunningham & Gotlieb 2005). The pro-oxidant milieu in intima creates an oxidative stress environment and promotes LDL cholesterol oxidation. Oxidized LDL (oxLDL) can further damage endothelial cells and induce adhesion molecules expression such as P-selectin and trigger monocytes and leukocytes migration into the intima (Napoli et al. 1997). Endothelial cell, smooth muscle cells and macrophage then secrete growth factors and inflammatory cytokines (Ross 1993). In intima, monocytes differentiate into macrophage and internalize oxLDL via scavenger receptor SR-A and CD-36 (Kunjathoor et al. 2002). This process eventually leads to foam cells formation and stimulate smooth muscle cells to proliferate and form a cap on top of the plaque. In some cases, plaques become unstable due to degradation of collagen by matrix metalloproteinase (MMPs) and prone to rupture (Lin et al. 2014) resulting in formation of thrombus (Figure 1).

Atherosclerotic plaque development involves the interaction between many genes and the environment. Potential genes that attribute to the heritability component include several mutations that mostly affect HDL and LDL cholesterol levels such as LDLR, ApoB, ApoA1 and ABCA1 (Watkins & Farrall 2006). Moreover, understanding the role of proteins that are important in inflammatory reaction such as VCAM-1, ICAM-1, IL-6 and MCP-1 and regulatory process such as NF- $\kappa$ B, TNF- $\alpha$  and IFN- $\gamma$ , are essential as these genes and proteins have potential to become biomarkers for pCAD. Assessment of their function needs to be done carefully as some of them are capable of executing multiple actions (Ozaki & Leonard 2002).

#### POTENTIAL BIOMARKERS FOR PCAD

Recently, the American College of Cardiology and the American Heart Association (ACC/AHA) has proposed a guideline for risk assessment for CAD (Goff et al. 2014). However, the recommendations did not emphasize on pCAD and only provide a rough estimation for individual risk. A number of studies also have proposed different types



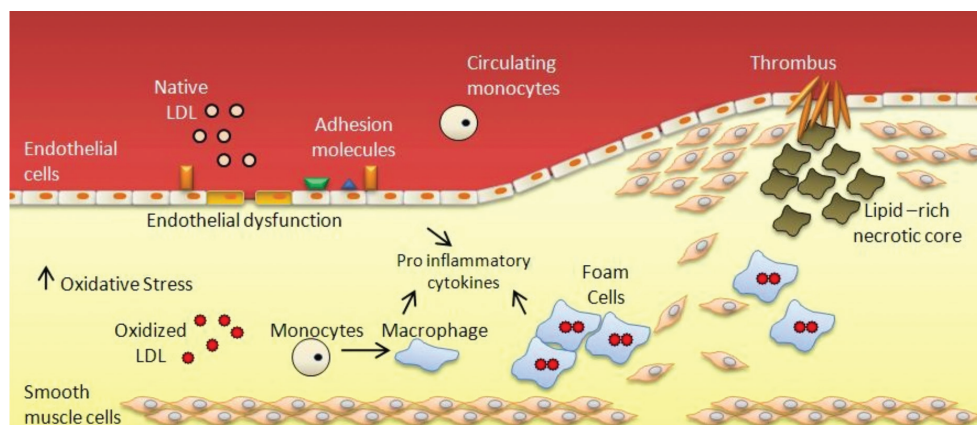


FIGURE 1. Mechanism of atherosclerosis. Endothelial dysfunction promotes migration of LDL particle into the sub-endothelial space where they are subjected to oxidation by reactive oxygen species. Oxidized LDL further trigger endothelial cells to express adhesion molecules including VCAM-1 and ICAM-1 and promote monocytes migration. In the sub-endothelial space, macrophages internalize oxidized LDL and form foam cells. This process involves multiple components including pro-inflammatory cytokines such as IL-6 and TNF- $\alpha$  produced by endothelial cells, macrophages and foam cells. Pro-inflammatory cytokines induce smooth muscle cells migration and form a cap on top of the plaque. In some cases, the plaque become unstable and prone to rupture and leads to thrombus formation

of biomarkers for CAD (Siemelink & Zeller 2014; Yayan 2013) while the importance of finding indicators for pCAD are fairly neglected. As some studies provide evidence that there are differences exist in the plaque morphology between younger and older CAD patients (Ruiz-Garcia et al. 2012), it is possible that current proposed biomarkers for CAD are not applicable for pCAD. Therefore there is an urgent need for studies that include risk assessment for young individuals and to develop biomarkers that are specific for this population.

#### HOMOCYSTEINE

An elevated serum concentration of homocysteine (a state called hyperhomocysteinemia) is a known risk factor for atherosclerosis and associated with an increased risk of myocardial infarction and mortality. An epidemiological study suggested a positive association between homocysteine (Hcy) concentrations and risk for cardiovascular disease (Graham et al. 1997). Previous data have shown that elevated homocysteine levels can be detected in 30% of CAD patients. The mechanisms involved by which homocysteine impairs vascular function is only partially understood. High homocysteine is involved in several potential mechanisms resulting in a decrease in the availability of endothelial cell-derived NO, impairment of endothelial function, an increment in monocyte adhesion (Faraci 2003), induction of oxidative stress, and increased tendency for thrombosis (Kaul et al. 2006). Although most available data support the involvement of homocysteine in CAD, most intervention studies which involved lowering homocysteine levels failed to reduce clinical events (Albert et al. 2008, Armitage et al. 2010).

The association of homocysteine with atherosclerosis in both case control observations and prospective cohort studies has been reported for pCAD. A case control study among male and female patients aged below 45 years with

CAD showed hyperhomocysteinemia as an independent risk factor for pCAD where the risk is higher in men (OR= 2.54, 95% CI, 1.23-5.22,  $p=0.01$ ). Hyperhomocysteinemia is shown to be significantly influenced by vitamin B12 deficiency (Sadeghian et al. 2006). A more recent study reported higher plasma homocysteine level in cases compared to healthy controls ( $22.14 \pm 10.62 \mu\text{mol/l}$  vs  $17.38 \pm 8.46 \mu\text{mol/l}$ ) with OR= 1.93 (95% CI, 1.27-2.94) (Gupta et al. 2012). Homocysteine levels also appear to be higher in multiple vessel disease. The mean homocysteine levels are 12.6 and 15.5  $\mu\text{mol/L}$  in patients with single and multi-vessel CAD respectively (Eftychiou et al. 2012). However, several studies reported contradicting findings. A cross sectional study showed neither high homocysteine nor lipoprotein (a) is associated with CAD in young women. However, a strong association with disease is found when both risk factors are present (OR=4.83,  $p=0.003$ ). Homocysteine level and lipoprotein (a) are also independent risk factors for CAD in young men. The presence of both risk factors did not confer additional risk to the development of pCAD (Foody et al. 2000).

#### APOLIPOPROTEIN

The metabolism of apolipoprotein is closely associated with the development of atherosclerosis. In particular, apoA1 is the main component in high density lipoprotein (HDL) involved in reverse cholesterol transport (Yancey et al. 2003), whereas ApoE is a protein that mediates the transport and uptake of cholesterol and lipid (Saito et al. 2004). Furthermore, ApoB is a protein constituent of the atherogenic very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and LDL (Ray et al. 2009). Measurement of plasma apolipoprotein allows an assessment of the total atherogenic and anti-atherogenic particles in the circulatory system.

Patients with high ApoB and small density LDL have been reported to have three-fold increased risk of pCAD (St-Pierre et al. 2005). In genetic dyslipidemia, reduced ApoA1 levels and elevated ApoB levels predict pCAD (Ayyobi et al. 2007). A study of 203 pCAD patients examined the association between ApoB and ApoA1 with pCAD. ApoA1 is found to be independently associated with pCAD in men and plasma ApoB level is the strongest independent variable associated with pCAD in women (Kwiterovich et al. 1992). A similar finding was also reported involving 243 men and 61 women with pCAD and 203 age and sex-match controls. ApoB level has been found to be a predictor of risk in women (Weber et al. 1997). A study in non-diabetic pCAD patients showed that ApoB is a better marker than traditional lipids where ApoB concentrations is significantly higher in CAD group compared to normal ( $102 \pm 24$  versus  $84 \pm 17$ ,  $p < 0.001$ ) (Azizi et al. 2002). Therefore, measurement of ApoA1 and ApoB levels may improve our ability to evaluate pCAD risk beyond lipid measurement.

#### FIBRINOGEN AND FIBRIN

Fibrinogen is the precursor of fibrin and considered to be a determinant for atherogenesis (Herrick et al. 1999). Multiple studies showed significant associations between CHD as well as cardiovascular disease (CVD) (Okwuosa et al. 2013). Fibrinogen circulates in the plasma and the normal concentration is 200-400 mg/dL. However, fibrinogen levels can decline with lifestyle modifications such as exercise (Ernst 1993) and smoking cessation (Hunter et al. 2001). In young adults, fibrinogen also showed a positive association with most risk factors for cardiovascular disease. Fibrinogen level is positively associated with body mass index, cigarette smoking, LDL cholesterol, blood pressure and triglycerides while negatively associated with HDL cholesterol, alcohol intake and physical activity (Folsom et al. 1993).

The role of fibrin in pCAD was examined in a group of young, post myocardial infarction patients with angiographic proven CAD and in healthy volunteers matched for age and sex. Young CAD patients had significantly higher plasma fibrinogen, plasminogen activator inhibitor type I, von Willbrand factor and lipoprotein (a) as compared to controls. Fibrin of young patients was made of numerous and shorter fibres, stiffer and lysed at a slower rate than controls. Fibrin stiffness was found to be an independent predictor for both hypofibrinolysis and pCAD (Collet et al. 2006). High plasma fibrinogen also predicts critical coronary lesions in pCAD (Tatli et al. 2009). A case-control study conducted to discover the association between fibrinogen and premature myocardial infarction in middle age men less than 55 years old showed an elevated level of plasma fibrinogen in patients ( $354.9 \pm 60$  mg/dL) compared to the control group ( $329 \pm 73$  mg/dL). Hyperfibrinogenemia was found in 81.8% of the patients and 57.5% of controls (OR= 3.3,  $p = 0.036$ ) (Shojaie et al. 2009). Essentially, in first degree

relatives of patients with pCAD, a significant increase in fibrinogen levels has been demonstrated. These findings highlighted the importance of screening fibrinogen in healthy individuals who other than their family history, appeared to be at low risk in terms of conventional CAD risk factors (Mills et al. 2002).

#### GAMMA GLUTAMYL TRANSFERASE (GGT)

GGT is an enzyme present in the serum and on the outer surface of cells. It is responsible for the synthesis of extracellular glutathione (Shabbir et al. 2011). GGT levels are examined for liver and gallbladder diagnosis, especially for alcoholic related disease. Recent evidence suggests that increased serum gamma glutamyl transferase (GGT) is associated with cardiovascular disease (Mao et al. 2014). A number of epidemiological studies partly explained the correlation of GGT with multiple risk factors including obesity, hypertension, diabetes and metabolic syndrome suggesting it might participate in oxidative and inflammatory reactions (Lee et al. 2003; Nakanishi et al. 2004; Onat et al. 2012). Relationship between GGT activity and cardiovascular incidents had also been shown to be independent of alcohol intake in a meta-analysis (Fraser et al. 2007). However, the exact mechanism that relates GGT and cardiovascular disease is still unclear. Several hypotheses have been proposed including GGT itself is pro-atherogenic (Emdin et al. 2005) and elevated GGT is a marker for metabolic syndrome (Lee et al. 2007).

In established CAD patients, GGT is independently associated with increased arterial stiffness in both men and women (Zhu et al. 2013). Moreover, the increased GGT activity is related to clinical stability, LV function and inflammatory activity rather than the CAD severity (Demircan et al. 2009). Shabbir et al. (2011) reported the association of GGT activity with pCAD in young Pakistani patients (age < 45 years old) who went for angiography. Their results showed that GGT activity was positively correlated with higher blood pressure, glucose, smoking and cholesterol and negatively correlated with antioxidant status. Furthermore, GGT activity increased significantly in pCAD compared to controls. Their findings suggest a good diagnostic accuracy at cut-off of 35 U/L, with 81% specificity, 92% sensitivity and diagnostic odds ratio of 48 for prediction of pCAD in young Pakistanis.

#### SYMMETRIC AND ASSYMETRICDIMETHYLARGININE (SDMA AND ADMA)

Assymetricdimethylarginine (ADMA) is an endogenous inhibitor for nitric oxide synthase and regulates the rate of NO production (Leiper & Vallance 1999). ADMA has been regarded as a potential biomarker for cardiovascular disease and may potentially influence restenosis after angioplasty (Derkacz et al. 2011). Moreover, among young healthy subjects, ADMA has been suggested to have a role in regulating systemic vascular tone (Paiva et al. 2008). Studies on ADMA associated risk in smokers and non-smokers showed an adjusted relative risk for future

coronary events of 2.40 (95% CI 1.14-5.08;  $p=0.021$ ) in non-smokers and 0.48 (95% CI 0.16-1.46;  $p=0.198$ ) in smokers. It was suggested that ADMA metabolism may be altered by tobacco smoke (Maas et al. 2007). A follow-up study involving 1148 coronary heart disease patients showed ADMA is not associated with secondary CVD events. After 8 years, 150 patients had a secondary event and 121 patients died and an association was only suggested for symmetric dimethylarginine (SDMA) (HR 1.17 [1.00–1.37]) (Siegerink et al. 2013). SDMA levels also have been shown to be independently associated with increased cardiovascular and all-cause mortality in subjects undergoing coronary angiography (Meinitzer et al. 2011). The differences in terms of the risk pattern in SDMA and ADMA suggest both methylarginines have unique pathophysiological roles in CAD.

A study on a population of young Indians (age between 15 and 50 years) showed plasma ADMA concentrations in patients with ischemic stroke were significantly higher compared to age matched controls (1.49 vs. 0.97  $\mu\text{mol/l}$ ,  $p<0.001$ ). The association with stroke remained significant even after adjustment with cardiovascular risk factors (OR=1.55, 95% CI 1.25–1.92,  $p<0.001$ ) (Mamatha et al. 2011). In another study on young Egyptians (age between 35 and 50 years old), only SDMA level is significantly elevated in patients. However, when comparing between chronic (patients who went for medical treatment, PCI or CABG) and acute (patients with acute myocardial infarction) patients, both ADMA and SDMA levels were significantly higher only in acute patients (Gad et al. 2010).

#### GENETIC POLYMORPHISMS AS BIOMARKERS

The development of atherosclerosis involves both environmental and genetic factors. Beside traditional risk factors such as smoking, physical activity and dietary, clinical outcome can be influenced by genetic variants. Recent genome-wide association studies have reported 35 common genetic variants associated with CAD in European population (Peden & Farrall 2011) which were replicated in Japan and Korean populations (Lee et al. 2013). Although the main contribution for these genome-wide scans is to identify possible mechanisms and pathways leading to the disease thus offering potential intervention, the usefulness of the SNPs as predictive biomarkers has been demonstrated in a few studies (Kathiresan et al. 2008; Ripatti et al. 2010). Several SNPs also showed significant association with pCAD providing further support of the potential of SNPs for pCAD (Table 2).

A previous study reported the association of 48 coding and 3 non-coding SNPs from 35 inflammatory genes with CAD. Significant association was observed between three locus haplotype in interleukin 1 cluster with  $p=0.006$  in all CAD cases,  $p=0.01$  in myocardial infarction and  $p=0.0002$  in pCAD (Brown et al. 2010). After adjusting for traditional risk factors including sex, smoking, hypertension and hypercholesterolemia, the haplotype effect remained significant in pCAD cases. A

cross-sectional, observational study conducted in pCAD patients and controls with a mean age below 45 years to determine the effect of IL-10 gene promoter polymorphism showed no relationship between polymorphism and adverse angiographic and clinical outcome, but found a significant difference in IL-10 -592C/A allelic frequency (OR=2.00, 95% CI 0.9434-4.2579) between patients and match controls (Karaca et al. 2011). However, in contrast, Cardiovascular Risk in Young Finns Study reported an inverse association in arterial stiffness index ( $p=0.0034$ ) and Young's elastic modulus ( $p=0.0005$ ) demonstrating discordance with the supposed anti-atherogenic properties of IL-10 (Heiskanen et al. 2010).

Similarly, IL-6 polymorphisms also showed conflicting findings. A study of Pakistani families involving 36 patients with mean age of  $46.4 \pm 18.7$  showed IL-6 polymorphism at -174 was more prevalent in CAD cases compared to healthy controls. There was significant association between IL-6 polymorphism and CAD suggesting an association with an increased risk for the disease (Satti et al. 2013). In multi-ethnic comparison, the -174 IL-6 allele frequency was higher in young Indians (23%) compared to Black participants (2%) with  $p<0.0001$  (OR = 0.05, 95% CI 0.018–0.14). A significant association with CAD has also been found among young Indian patients when compared to control. However, several studies found no association of IL-6 with CAD (Ghazouani et al. 2011; Sekuri et al. 2007). Nevertheless, single nucleotide polymorphism may offer predictive value for predisposition to pCAD.

#### MICRO-RNA (MIRNA)

Small non coding RNAs (miRNA) are typically 20 to 26 nucleotides long which function mainly in regulation of post-transcriptional gene expression. As such, miRNA plays multiple roles in preserving biological processes including cell proliferation, apoptosis and tumorigenesis (Cordes & Srivastava 2009). In recent years, interest has escalated on miRNA as a potential biomarker as well as a potential therapeutic approach for cardiovascular disease (De Rosa et al. 2014; Hagiwara et al. 2014; Olson 2014). Numerous studies have been conducted on circulating miRNA in either acute myocardial infarction or CAD (Table 3). For instance, 11 miRNAs have been found significantly downregulated among CAD patients compared to healthy controls. Although medication used may alter the relative miRNA expression, these results have implications in the use of miRNA for identifying and managing individuals with CAD or at higher risk of developing disease (Weber et al. 2011).

A case control study investigated the role of miRNA in pCAD by comparing relative expression levels of platelet miRNAs using microarrays (Sondermeijer et al. 2011). Six platelet miRNAs were significantly upregulated in pCAD (miR340, miR451, miR454, miR545:9.1, miR615-5p and miR624) while one miRNA (miR1280) was downregulated compared to controls. Validation studies in

TABLE 2. Association of several SNPs with pCAD from previous studies

SNPs	Gene	Non-risk allele	Risk allele	Genotype / Allelotype	Finding Odd Ratio (Confidence Interval), p value	Population	Function	Ref
rs10757278	9p21.3	A	G	GG G allele	2.08 (1.19–3.66), p= 0.006 1.49 (1.08–2.07), p= 0.011	<ul style="list-style-type: none"> <li>Polish Caucasians</li> <li>160 pCAD, 160 controls</li> <li>Mean age (cases) = 41.21–5.62</li> </ul>	The G allele disrupts a binding site for STAT1 which is an interferon- $\gamma$ -activated transcription factor	Niemiec et al. (2012)
rs9932581	CYBA	A	G	GG + AG	2.03 (1.21–3.44), p= 0.007	<ul style="list-style-type: none"> <li>240 pCAD, 240 blood donors</li> <li>mean age (cases) 44.55 <math>\pm</math> 6.09</li> </ul>	It influence CYBA transcriptional activity by modulating CYBA promoter activity	Niemiec et al. (2014)
rs1800795	IL-6	C	G	G allele	0.47 (0.23–0.95), p= 0.043	<ul style="list-style-type: none"> <li>South African Indian</li> <li>100 pCAD, 100 healthy controls</li> <li>mean age(cases) = 37.5</li> </ul>	G allele may influence IL-6 levels and promote inflammation during CAD progression	Phulukdaree et al. (2013)
rs1799983	eNOS	G	T	GT TT	1.7 (0.95–2.9), p= 0.09 2.6 (1.2–5.7), p= 0.025	<ul style="list-style-type: none"> <li>Egyptian</li> <li>116 pCAD, 119 controls</li> <li>Mean age (cases) = 42.4 <math>\pm</math> 7.3</li> </ul>	This polymorphism maybe associated with CAD through decreased NO bioavailability	Abdel & Mohamed (2013a)
rs1800947	CRP	G	C	GC + CC C allele	2.02 (0.97–4.20), p= 0.08 1.99 (0.99–4.02), p= 0.07	<ul style="list-style-type: none"> <li>Egyptian</li> <li>116 pCAD, 119 controls</li> </ul>	Mean age(cases) = 42.4 $\pm$ 7.3 It has been suggested to effect CRP mRNA stability or act through linkage disequilibrium with nearby genetic polymorphisms	Abdel & Mohamed (2013b)



TABLE 3. MicroRNA expressions in CAD or MI cases

MicroRNA	Sample Population	Sample Type	Finding	Method	References
miR-133a	13 AMI, 176 angina pectoris patients, 127 controls	Plasma	<ol style="list-style-type: none"> <li>1. Circulating miR-133a significantly increase in AMI patients in time dependent manner</li> <li>2. miR-133a had diagnostic accuracy for CHD with AUC of 0.918</li> </ol>	qRT-PCR	Wang et al. (2013)
miR-526b	12 CAD patients, 12 age-matched controls	Not specified	<ol style="list-style-type: none"> <li>1. MiR-526b was significantly upregulated in CAD patients</li> </ol>	Array	Li et al. (2014)
miR-135a miR-147	25 stable angina pectoris patients, 25 unstable angina patients, 20 healthy controls	PBMC	<ol style="list-style-type: none"> <li>1. Mir-135a expression increase 5-fold compared to controls (p&lt;0.001)</li> <li>2. miR-147 decrease 4-fold compared to controls (p&lt;0.01)</li> </ol>	qRT-PCR	Hoekstra et al. (2010)
miR-126	31 patients with CAD, 31 controls	Plasma	<ol style="list-style-type: none"> <li>1. Mir-126 was not differentially expressed in CAD patients when compared to controls</li> </ol>	qRT-PCR	Sun et al. (2012)
miR-1	93 AMI patients, 66 healthy subjects	Plasma	<ol style="list-style-type: none"> <li>1. Mir-1 levels significantly higher in AMI patients compared to healthy subjects.</li> <li>2. The levels dropped to normal upon discharge following medication</li> </ol>	qRT-PCR	Ai et al. (2010)
miR-214	12 subjects with stable angina pectoris, 16 with unstable angina pectoris and 12 with AMI, and 15 controls without CAD	Plasma	<ol style="list-style-type: none"> <li>1. MiR-214 level was significantly lower in CAD patients compared in controls (P &lt; 0.01)</li> <li>2. Decrease level of miR-214 may lead to increased PLGF levels and promote atherosclerosis</li> </ol>	qRT-PCR	Lu et al. (2013)
miR-210, miR-451, miR-150, miR-186, miR-1 and miR-133a/b	24 AMI patients, 8 healthy adults	Heart Tissue	<ol style="list-style-type: none"> <li>1. MiR-210, miR-451, miR-150 and miR-186 up-regulated in MI</li> <li>2. MiR-133a/b down-regulated in MI</li> <li>3. Mir-1 upregulated in remote tissues but not in infarcted tissues</li> </ol>	Array qRT-PCR	Bostjancic et al. (2010)
miR-494, miR-490-3p, and miR-769-3p	34 CAD patients, 49 nonCAD subjects	Plasma	<ol style="list-style-type: none"> <li>1. MiR-494, miR-490-3p, and miR-769-3p expressions were significantly higher in CAD</li> <li>2. hsa-miR-769-3p was significantly correlated with the presence of CAD</li> </ol>	qRT-PCR	Freedman et al. (2012)
miR-19a, miR-584, miR-155, miR-222, miR-145, miR-29a, miR-378, miR-342, miR-181d, miR-150, and miR-30e-5p	<ol style="list-style-type: none"> <li>1. 5 CAD subjects and 5 healthy controls (array)</li> <li>2. 10 CAD subjects and 15 healthy controls (qRT-PCR)</li> </ol>	Whole Blood	<ol style="list-style-type: none"> <li>1. miRNA levels in blood were low and microarray data unable to give adequate informations</li> <li>2. qRT-PCR data showed miR-19a, miR-584, miR-155, miR-222, miR-145, miR-29a, miR-378, miR-342, miR-181d, miR-150, and miR-30e-5p were significantly downregulated in CAD subjects compared to controls</li> </ol>	Array qRT-PCR	Weber et al. (2011)



two different cohorts confirmed that miR340 and miR624 were upregulated in pCAD. However the limitation of this study is that the differences in miRNA expression levels between patients and controls were very small. It is difficult to determine if both miRNAs can be used as independent biomarkers for pCAD. Taken together, miRNAs have the potential utility to be novel biomarkers in CAD particularly in younger adults. However, further studies conducted using larger cohorts are necessary to evaluate and prove their candidacy as predictors for pCAD.

#### CONCLUSION

Understanding the mechanism of atherogenesis including lipid metabolism, endothelial dysfunction, oxidative stress and inflammation may be crucial if prevention strategies are to be targeted accurately. The usual risk factors of age, sex, family history, cholesterol level, smoking status, blood pressure and diabetes may be insufficient to predict risk to pCAD. The emergence of omics technology offer extensive information on the genes and proteins involved during disease progression and as such, can be potential biomarkers for CAD. As protein markers give an insight on the actual body condition, genetic markers might be a better marker for risk assessment. Knowledge at the genome level such as polymorphism can enhance our ability to identify high risk individuals as genomic data provide the information on susceptibility to disease particularly pCAD. A well-designed clinical trial should be conducted in order to properly validate their candidacy and maximize their potential as biomarkers.

A good biomarker should be highly sensitive and specific to be used clinically. Although many biomarkers for CAD such as homocysteine, IL-6 or ApoB/ApoA1 ratio have been proposed, none are routinely used in diagnostic procedure except for CRP. Perhaps one of the reasons is because of evidence insufficiency from prospective cohort studies. As CAD is a complex disease that involves multiple stages and interactions, relying on one biomarker might be inadequate. The ideal alternative is to introduce a panel of biomarkers that provide a risk score or algorithm and evaluate the predisposition to pCAD so that early preventive measures and lifestyle modifications can be done. Nevertheless, recent data on potential biomarkers for pCAD can provide the basis for studies to further justify their prognostic value. Future studies should take the next step for risk prediction including in the determination of their role during disease progression as well as establishing markers to be used routinely.

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Muhammad Faizan A. Shukor\*, Noor Akmal Shareela Ismail & Wan Zurinah Wan Ngah  
Department of Biochemistry  
Faculty of Medicine, UKM Medical Centre  
Universiti Kebangsaan Malaysia  
Jalan Ya'acob Latif, Bandar Tun Razak  
50300 Kuala Lumpur, Federal Territory  
Malaysia

Muhammad Faizan A. Shukor\*  
Centre for Chemical Defense  
Universiti Pertahanan Nasional Malaysia (UPNM)  
Kem Sungai Besi  
57000 Kuala Lumpur, Federal Territory  
Malaysia

\*Corresponding author; email: mfaizanshukor@gmail.com

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