EXPRESSION PATTERNS OF MARKERS OF INFLAMMATION, ENDOTHELIAL DYSFUNCTION AND PLATELET ACTIVATION IN EARLY PHASE OF ACUTE CORONARY SYNDROME

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ABSTRACT

(Inflammation, endothelial dysfunction and platelet activation play an important role in pathogenesis of vulnerable plaques of acute coronary syndrome (ACS). However little is known about the inter-relation of these indices in early phase of ACS. This study sought to determine a possible multimarker strategy by linking these three indices to provide an early assessment of the risk of ACS. Serum protein and gene expression level of interleukin-6 (IL6; biomarker of inflammation), von Willebrand Factor (VWF; biomarker of endothelial dysfunction) and soluble P-selectin (SELP; biomarker of platelet activation) were measured in 22 patients with ACS (age 55.2±10.4 years) and 28 controls with angiographically documented occlusive coronary artery disease (CAD) without previous ACS events (age 53.5±8.4 years). Venous blood from ACS patients was obtained within 30 minutes of hospital admission. In this study, the gene expression of IL6, VWF and SELP by using total RNA extracted from whole blood was successfully examined. ACS patients had significantly higher serum level of IL6 and VWF levels (p < 0.001), compared to controls. However, such increase levels were not reflected in gene expression levels of IL6 (p = 0.536) and VWF (p = 0.227), which we believed might be due to post-translational modification. Moreover, no significant difference in SELP serum (p = 0.082) and gene expression level (p = 0.675) between ACS patients and controls was observed. Nevertheless, a strong correlation was found between SELP and IL6 (r = 0.697, p = 0.003) and between SELP and VWF (r = 0.497, p = 0.05) at gene expression levels which indicated the possible association between these three indices in pathogenesis of ACS. The results of this study add to the current knowledge of molecular pathogenesis of early phase of ACS by demonstrating the association of inflammation, endothelial dysfunction and platelet activation marker. During early hospital
presentation with ACS in a multiethnic Malaysian population, inflammation and endothelial
dysfunction play a more prominent role in the pathogenesis of the disease, and IL6 and VWF
can be used together as plaque instability markers to provide an early assessment of the risk
of ACS.

Keywords: Acute coronary syndrome, interleukin-6, von Willebrand Factor, P-selectin
EKSPRESI POLA PENANDA BUKU INFLAMASI, DISFUNGSI ENDOTEL DAN PENGAKTIFAN TROMBOSIT PADA FASA AWAL SINDROM KORONARI AKUT

ABSTRAK

Inflamasi, disfungsi endotel dan pengaktifan platelet memainkan peranan penting dalam patogenesis plak terdedah dalam sindrom koronary akut (acute coronary syndrome, ACS). Namun, sedikit yang diketahui tentang keterkaitan indeks-indeks ini dalam peringkat awal ACS. Kami ingin menentukan satu strategi multi penanda yang dapat menyambung ketiga-tiga indeks ini untuk membeberkan awal risiko ACS. Tahap serum protein dan gen ekspresi interleukin-6 (IL6; penanda inflamasi), von Willebrand Factor (VWF; penanda disfungsi endotel) dan soluble P-selectin (SELP; penanda pengaktifan platelet) diukur dalam 22 orang ACS pesakit (umur 55.2±10.4 tahun) dan 28 orang kawalan (umur 53.5±8.4 tahun) yang diketahui penyakit oclusif arteri koronari (Coronary artery disease, CAD) selepas kajian angiographic dan tanpa acara ACS sebelumnya. Darah vena diperohi daripada pesakit ACS dalam masa 30 minutes selepas persembahan ke hospital. Dalam kajian ini, ekspresi gen IL6, VWF dan SELP berjaya diperiksakan dengan menggunakan total RNA diekstrakan dari seluruh darah. Pesakit ACS didapati memiliki lebih tinggi peringkat serum IL6 dan VWF (p < 0.001) berbanding dengan pesakit kawalan. Namun demikian, kenaikan tersebut tidak tercermin dalam tahap ekspresi gen IL6 (p = 0.536) dan VWF (p = 0.227), dengan itu kami percaya ini mungkin disebabkan daripada modifikasi pasca-translasi. Selain itu, tidak ada perbezaan yang signifikan diperolehi dalam peringkat serum (p = 0.082) dan gen ekspresi (p = 0.675) SELP di antara pesakit ACS dan kawalan. Namun, satu korelasi yang kuat ditemui antara SELP dan IL6 (r = 0.697, p = 0.003) dan antara SELP and VWF (r = 0.497, p = 0.05)
pada tahap ekspresi gen yang menunjukkan kemungkinan hubungan antara ketiga-tiga indeks ini dalam patogenesis ACS. Keputusan kajian ini menambah pengetahuan kami dalam patogenesis molekul fasa awal ACS dengan menunjukkan hubungan antara penanda inflamasi, disfungsi endotel dan pengaktifan platelet. Selama persembahan awal ke hospital dengan ACS dalam populasi multiethnik Malaysia, inflamasi dan disfungsi endotel memainkan peranan yang lebih penting dalam patogenesis penyakit, dan IL6 dan VWF boleh digunakan bersama sebagai penanda ketidakstabilan plak untuk memberikan penilaian awal risiko ACS.

Kata kunci: Sindrom koronari akut, interleukin-6, von Willebrand Factor, P-selectin
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<tr>
<td>ΔΔCT</td>
<td>Delta-delta C_T</td>
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<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>ACTB</td>
<td>Human β-actin gene</td>
</tr>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>BRNA</td>
<td>Human Breast Total RNA</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complimentary DNA</td>
</tr>
<tr>
<td>CK-MB</td>
<td>Creatinine kinase, muscle and brain</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>C_T</td>
<td>Threshold cycle</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>CVRF</td>
<td>Cardiovascular risk factor</td>
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<tr>
<td>dNTP</td>
<td>Deoxyribonucleotide triphosphate</td>
</tr>
<tr>
<td>E</td>
<td>Real time PCR efficiency</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
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<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>GRACE</td>
<td>Global Registry of Acute Coronary Event</td>
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<tr>
<td>GOI</td>
<td>Gene of interest</td>
</tr>
<tr>
<td>GPIIb/IIIa</td>
<td>Glycoprotein IIb/IIIa</td>
</tr>
<tr>
<td>GPV1</td>
<td>Glycoprotein V1</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intracellular adhesion molecule 1</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>IL6</td>
<td>Interleukin 6</td>
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<tr>
<td>Inter-CV</td>
<td>Inter-assay CV</td>
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<tr>
<td>Intra-CV</td>
<td>Intra-assay CV</td>
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<tr>
<td>IQR</td>
<td>Interquartile ranges</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>MMLV</td>
<td>Moloney Murine Leukemia Virus</td>
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<tr>
<td>MOH</td>
<td>Ministry of Health</td>
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<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
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N.A.  
Not available

NMRR  
National Medical Research Register

NO  
Nitric Oxide

NSTEMI  
Non ST-segment elevation myocardial infarction

NTC  
Non-template control

p  
Significance difference

PCM  
Pericellular matrix

PCR  
Polymerase chain reaction

$r^2$  
Pearson/Spearman correlation coefficient

RT  
Reverse transcription

stdev  
Standard deviation

STEMI  
ST-segment elevation myocardial infarction

TF  
Tissue factor

$T_m$  
Melting temperature

TNF-α  
Tumour necrosis factor alpha

UA  
Unstable angina

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<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>VSMC</td>
<td>Vascular smooth muscle cells</td>
</tr>
<tr>
<td>v.s.</td>
<td>Versus</td>
</tr>
<tr>
<td>VWVF</td>
<td>Von Willebrand Factor</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight/volume</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cells</td>
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CHAPTER 1

INTRODUCTION

Coronary artery disease (CAD), including its manifestation as acute coronary syndrome (ACS) is a well-established cause of morbidity and mortality among adults in both developed and developing countries (Husten, 1998; Chin et al., 2008). In recent years, there is an alarming rise in the incidence of ACS in Malaysian populations especially in younger age groups less than 55 years old (Wan Azman and Sim, 2006).

A disruption of an atherosclerotic plaque with superimposed thrombosis had been identified as the main cause of ACS, including acute myocardial infarction (AMI), unstable angina (UA) and sudden death (Libby and Theroux, 2005; Char, 2005). The process of attributing atherosclerosis could lead to the concept of Birmingham’s vascular triad. The initial damages of endothelium walls prompted by conventional cardiovascular risk factors (CVRF); followed by a phase of plaque development toward an increasing likelihood of plaque rupture and thrombosis can eventually cause ACS. This model hypothesises the roles of three phases, namely “atherogenesis”, “angiogenesis” and “thrombogenesis”, together with endothelial damage and CVRF explains the possible underlying pathogenesis of ACS (Makin, 2002; Chung and Lip, 2003/2004; Lip and Blann, 2004).

A biomarker, which may be a target gene or protein product representing a particular phase of disease mechanism has gain a lot of attention in clinical researches recently (Apple et al., 2005; Vasan, 2006). These biomarkers may prove useful in the goal of developing a “predictive, pre-emptive, personalized tool” to risk stratify individuals for prognosis and potential intervention for ACS (Panteghini, 2004; Morrow and de Lemos, 2007). Currently available diagnostic tools of ACS, including myocardial markers such as troponin-T, CK-MB
or myoglobin have several shortcomings. These include poor specificity and delayed sensitivity for timely detection of myocardial necrosis. In addition, they only detect the myocardial damage phase after an ACS event has occurred, thus the information to identify patients at risk of ACS in early phase is not available (Panteghini, 2004; Morrow et al., 2007; Loria et al., 2008). Therefore the search for a method that can rapidly ascertain whether a patient has an ACS, even at his very early phase of disease, is one of the utmost priorities of modern emergency medicine (Sim et al., 2008).

It is found that inflammatory markers such as interleukin-6 (IL6) can indicate the severity of local inflammation and their abnormal serum levels, together with von Willebrand factor (VWF; endothelial dysfunction marker) and P-selectin (SELP; platelet activation marker) have been assessed individually in many studies and each found to be the independent risk factor to predict the risk of CAD (Ridker et al., 2000; Hillis et al., 2002; Ray et al., 2005). However, their linkage to each other in early phase of ACS still remains unknown.

To further understand the underlying pathogenesis of ACS, the present study proposes a hypothesis that relates these three markers with the Birmingham's vascular triad as a backbone model. In conjunction with this model, IL6 may represent an early inflammation event linked to progressive plaque rupture ("atherogenesis"), VWF indicates the endothelial damage and activity of SELP may related to thrombus formation ("thrombogenesis") in the development of ACS.

Whilst blood also plays critical roles in immune and hormonal responses in the body, it is believe that development of ACS can induce changes that are detectable in blood. This again, may provide a valuable diagnostic medicine of an individual or serve as a surrogate tissue for clinical researches as it can be collected with minimal effort and as an alternative
tissue if primary tissue is not available (Aziz et al., 2008). With that, new methodologies such as gene expression studies using whole blood sample and protein analysis of multimarker strategy were proposed to identify the relationship of IL6, VWF and SELP in underlying pathophysiological processes of ACS.

From a clinical perspective, identifying any integral relationship between inflammation, endothelial dysfunction and platelet activation markers which are believed to be involved in phases as suggested by Birmingham’s vascular triad model will not only enhance the understanding of underlying mechanisms of these conditions, but may potentially help to improve diagnosis and pinpoint treatment targets for patients, even at their very early phase of ACS.
2.1. Acute Coronary Syndrome (ACS)

Heart diseases and diseases of pulmonary circulation is the top cause of deaths in Malaysia, which accounts for 16.9% of overall death (MOH Malaysia, 2010). Among these, acute coronary syndrome (ACS) is found to be of relatively high morbidity and mortality (MOH Malaysia, 2001; Chin et al., 2008). ACS results from acute obstruction of a coronary artery due to plaque rupture and thrombogenesis (Ross, 1999). Depending on the size, location and duration of obstruction, the consequences of ACS can range from unstable angina (UA) to non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI) (Grech and Ramsdale, 2003).

2.1.1. Etiology of ACS

Acute coronary syndrome is the consequence of atherosclerosis of which is the cause of coronary artery, peripheral vascular and cerebral vascular diseases (Ross, 1999). Atherosclerosis may progress over time by building up of atheromatous plaque which made up of fatty deposits in the coronary arteries (Carter, 2005; Correale et al., 2008). These plaques can become unstable or vulnerable to rupture. When the plaque ruptures, the thrombogenic materials will be exposed thus activating the platelets and the coagulation cascade, which then produce an acute thrombus (Fareed et al., 1998; Grech and Ramsdale, 2003). The occlusion of thrombus within the lumen of artery is the leading cause of myocardial ischemic event that is clinically manifested as ACS (Figure 2.1).