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THE RELATIONSHIP BETWEEN C-REACTIVE PROTEIN LEVELS AND MORTALITY RISK

Disusun Oleh:
Hoirun Nisa, M.Kes., Ph.D
NIP: 19790427 200501 2 005
NIDN: 2027047901
(Fakultas Kedokteran dan Ilmu Kesehatan UIN Syarif Hidayatullah Jakarta)

ABSTRACT

Background. High-sensitivity C-reactive protein (hsCRP) levels are decreased in Asian compared with Western subjects. It is uncertain whether hsCRP is a potent predictor of mortality at lower CRP concentrations. The present study examined the associations of hsCRP with the risks of all-cause and cause specific mortality in Japanese population. The present study also examined the concentrations of hsCRP in Indonesian.

Methods. Subjects were 4,737 men and 6,343 women aged 49-76 years participating in the baseline survey of an ongoing cohort study of lifestyle-related diseases between February 2004 and July 2006. Hazard ratios for all-cause and cause-specific mortality associated with hsCRP levels were estimated using Cox proportional hazards regression. For hsCRP levels in Indonesian subjects, recorded hsCRP levels of patients in hospitals were referred to.

Results. A total of 436 all-cause deaths occurred during a median follow-up of 8 years. The main cause of death was cancer. In men, hsCRP levels were positively associated with the risk of all-cause mortality as well as deaths from cancer and cardiovascular disease (CVD). All-cause mortality hazards for the 2nd (0.34-0.84 mg/L) and the 3rd (≥ 0.85 mg/L) tertiles of hsCRP were 1.27 (95% confidence interval [CI], 0.93-1.73) and 1.75 (1.30-2.37) in men, respectively (p for trend=0.001). In women, increased risks of all-cause and cause-specific mortality associated with elevated hsCRP levels were observed, but the associations were not statistically significant. In Indonesian subjects, males had a higher mean CRP level compared to females.

Conclusions. HsCRP was an independent predictor of all-cause, cancer, and CVD mortality in apparently healthy Japanese men, but not women. The differential effect of hsCRP in predicting mortality risk by sex warrants further investigation. The mean CRP level in the Indonesian subjects was not too high compared to the level determined by the laboratory assessing the C-reactive protein level.

Keywords: C-reactive protein, mortality, cancer, cardiovascular disease, inflammation, general population.

CONTENTS

CHAPTER I INTRODUCTION

1.1. Background

Chronic low-grade inflammation can lead to the development of chronic diseases and increased mortality risk (De Martinis et al., 2006; Hotamisligil, 2006). C-reactive protein (CRP) is an acute phase protein synthesized by the liver that increases in response to systemic inflammation and is one of the most important inflammatory markers (Pepys and Hirschfield, 2003). CRP is acknowledged to be an established risk factor for cardiovascular disease (CVD) (Ridker, 2016), and has been associated with the risk of cancer development (Guo et al., 2013). Several prospective studies have demonstrated that elevated CRP levels were associated with increased risks of all-cause and CVD mortality in combined men and women (Arima et al., 2008; Zacho et al., 2010; Swede et al., 2014; Zuo et al., 2016), and some studies have suggested that the association of CRP with CVD mortality differed by sex (Ko et al., 2012; Ahmadi-Abhari et al., 2013; Doran et al., 2013). The findings on the relationship between CRP and cancer mortality are inconsistent. For example, multiple studies suggested a modest increase in the risk of overall cancer mortality associated with elevated CRP levels (Il'yasova et al., 2005; Allin and Nordestgaard, 2011), but a recent study failed to find an association (Zuo et al., 2016).

The information on the importance of CRP as a predictor of mortality is derived mainly from studies performed in Western populations (Kaptoge et al., 2010). Although Asian had a lower hsCRP level compared with Western subjects (Kaptoge et al., 2010), a meta-analysis based on four Japanese studies showed that elevated hsCRP levels were significantly associated with an increased risk of ischemic stroke (Saito et al., 2014). However, the evidence on the associations of hsCRP with mortality risks from a large population-based studies is rare among Asian subjects (Arima et al., 2008; Iso et al., 2009).

The present study was to evaluate the associations between hsCRP and the risk of all-cause mortality as well as deaths from cancer, CVD, and other diseases in a large Japanese population. This is the first study regarding hsCRP and cancer mortality in middle-aged and elderly men and women in Japan.

1.2. Objectives

The present study was to evaluate the associations between hsCRP and the risk of all-cause mortality as well as deaths from cancer, CVD, and other diseases in a large Japanese population. This is the first study regarding hsCRP and cancer mortality in middle-aged and elderly men and women in Japan. The present study will also compare the CRP level in Japan and Indonesia.

1.3. Problem Statement

High-sensitivity C-reactive protein (hsCRP) is an important inflammatory marker, and inflammation is known to be involved in the initiation and progression of cancer. Aside from whether measurement of hsCRP is useful in assessment of cancer risk, studies are needed to help find out if hsCRP is a mediator of cancer. Several studies have shown that C-reactive protein can be a predictor of cancer risk. However, a limitation of these studies is that a majority of the participants were white of European or North American ancestry. Therefore, the relevance of hsCRP in Asian populations remains unclear. Furthermore, powerful analyses are needed to find out whether the strength of association of hsCRP levels and mortality risk varies by age, sex, or other clinically relevant subgroups.

This study will answer the following research questions:

1. What is the mean of hsCRP level in Japan and Indonesia?
2. What are hsCRP levels according to age and sex?

3. What are hsCRP levels according to lifestyle factors (body mass index, smoking status, drinking status, exercise, morbidities, and clinical features)?
4. What are the hazard ratios of all-cause and cause-specific mortality by sex in Japanese subjects?

1.4. Contribution of the Study

The contributions of the study are as the following:

1. We contribute to the growing literature of hsCRP levels, especially in Asian population.
2. As a marker for chronic inflammation, hsCRP assists in the identification of subjects with increased risks of all-cause and cause-specific mortality.
3. The study adds to the knowledge of how hsCRP levels to predict mortality would alter therapy or improve outcomes of patients.

1.5. Scope of the Study

Associations of hsCRP with risk of major diseases can best be assessed by long-term prospective follow-up of large numbers of people. This study is conducted to examine the association between hsCRP levels and mortality from all-cause and cause-specific mortality. Data are collected through survey and laboratory measurement. The study subjects are men and women aged 49-76 years from the ongoing cohort study of lifestyle-related diseases between February 2004 and August 2007. Residents of the East Ward of Fukuoka City were invited to participate in the study by mail. All participants were informed of the details of the survey and gave written informed consent prior to their participation in the study. Previous study about serum CRP in Indonesia population will be reviewed to compare the mean of CRP in Japan and Indonesia.

The study was approved by the Ethics Committee of the Kyushu University Faculty of Medical Sciences. During the period from February 2004 to August 2007, a total of 12948 subjects participated in the baseline survey, with a participation rate of 24%. Age of the participants at the time of survey ranged from 49 to 76 years. Stored serum samples of 12942 participants were available for the measurement of CRP. At the baseline survey, each participant completed a self-administered questionnaire and underwent measurements of blood pressure, height (cm) and body weight (kg). Fasting or non-fasting venous blood was drawn. The questionnaire inquired about smoking habits, alcohol consumption, physical activity, dietary intake, disease history, and use of selected drugs and supplements. HsCRP levels of Indonesian subjects will be examined in terms of mean, using the data from hospitals.

CHAPTER II THEORETICAL FRAME

C-reactive protein (CRP), a plasma protein synthesized by the liver, is a sensitive and dynamic systemic marker of inflammation (Kushner I, 1982). Its concentration in the circulation can increase by up to 10 000-fold during acute responses to serious infection or major tissue damage.

2.1. Function

CRP binds to the phosphocholine expressed on the surface of dead or dying cells and some bacteria. This activates thecomplement system, promoting phagocytosis by macrophages, which clears necrotic and apoptotic cells and bacteria. This so-called acute phase response occurs as a result of a rise in the concentration of IL-6, which is produced bymacrophages as well as adipocytes in response to a wide range of acute and chronic inflammatory conditions such as bacterial, viral, or fungal infections; rheumatic and other inflammatory diseases; malignancy; and tissue injury and necrosis. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver (Pepys et al., 2003).

CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin-mediated phagocytosis), which express a receptor for CRP. It plays a role in innate immunity as an early defense system against infections. CRP rises within two hours of the onset of inflammation, up to a 50,000-fold, and peaks at 48 hours. Its half-life of 48 hours is constant, and therefore its level is determined by the rate of production and hence the severity of the precipitating cause. CRP is thus a screen for inflammation (Pepys et al., 2003).

2.2. CRP Levels

CRP is present in only trace amounts in healthy individuals, and is hardly detectable by the standard clinical tests, which typically have a lower detection limit of 3–8 mg/L. CRP concentrations of recent research interest are in the so called normal range which can be measured only by the high-sensitivity testing. The lower detection limit for high sensitivity CRP (hs-CRP) is as low as approximately 0.04 mg/L. In a study of healthy blood donors, the median of hs-CRP levels was 0.8 mg/L with a range of 0.07–29 mg/L. Serum amyloid A protein and fibrinogen are acute-phase reactants which also show a temporal profile similar to the change of CRP in acute inflammation. The reasons for the wide use of hs-CRP are stability and availability of robust, automated methods for its measurement (Pepys et al., 2003; Nanri et al., 2007).

Marsik et al., (2008) reported that patients with CRP concentrations >5 mg/L at the time of hospital admission had a 50% to 330% increase in risk of death from any cause. This increase in risk was present in both short-term and long-term follow-up, rose in magnitude as concentrations of CRP increased >10 mg/L, and was associated with not only cardiovascular mortality but also mortality from cancer. Importantly, the mortality risk associated with increased CRP concentrations was independent of underlying disease status or reason for initial hospitalization. Characterizing CRP as a unique “triage marker for future death,”

Marsik et al., (2008) argue that evaluation of inflammatory risk in the hospital setting should be routinely performed to identify very-high-risk patients in need of additional close monitoring. While provocative, this conclusion is consistent with data from prior investigations that have reported hs-CRP to predict total mortality among patients hospitalized for acute ischemia, patients in general intensive care units, those undergoing

bypass surgery, or in clinics limited to patients with diabetes, end stage renal failure, chronic obstructive pulmonary disease, or cancer (Ridker et al., 2008).

2.3. Behavioral Correlates of Circulating hsCRP

Many studies have shown a strong, positive association between obesity and hsCRP, and weight loss results in decrease in hsCRP concentrations. A causal link between obesity and CRP is also supported by recent laboratory evidence. Adipocytes and monocyte-derived macrophages in expanded adipose tissue mass secrete proinflammatory cytokines such as TNF-α and IL-6, and thereby enhancing hepatic synthesis of CRP Elevation of hsCRP is also noted in the presence of the metabolic syndrome, a constellation of metabolic abnormalities such as glucose intolerance, hypertriglyceridemia, and hypertension which increases the risk of cardiovascular disease as well as of type 2 DM, with central obesity as a core component. CRP levels have been shown to be progressively increased with increase in the number of abnormal metabolic features in different populations (Nanri et al., 2007).

Smoking is related to increased levels of hsCRP. This positive association may be a reflection of underlying atherosclerotic lesions or due to systemic or non-vascular local inflammation. It has been shown that circulating hsCRP is lower in both men and women with moderate alcohol consumption. In a cross-sectional study, CRP concentrations were lower with increasing levels of alcohol consumption with no further decrease at the highest levels of intake. Exceptionally, alcohol drinking was positively correlated with CRP levels in a cross-sectional study in Russia. It is notable that alcohol consumption is derived mainly from binge vodka intake in this study population. Lower concentrations of hsCRP have been reported in individuals with high physical activity and high physical fitness estimated by maximal oxygen uptake. Obesity and smoking are undoubtedly related to high concentrations of hsCRP. Although further studies are needed as to the relation of alcohol and physical activity to hsCRP, it is obviously necessary to take into consideration these factors in

investigating the relation to disease risk as well as dietary determinants of hsCRP (Nanri et al., 2007).

2.4. Elevated CRP Levels and Disease Risk

2.4.1. Atherosclerotic diseases

Raised concentrations of total and low-density lipoprotein (LDL) cholesterol are an important risk factor for atherosclerotic diseases. It is well recognized that inflammation links to the development and progression of atherosclerosis. The initial step is endothelial cell activation, probably triggered by modified LDL, cigarette smoking, hypertension, hyperglycemia, and infectious microorganisms such as *Chlamydia pneumoniae* and Herpes viruses. The endothelial activation is characterized by expression of monocyte adhesion molecules and chemotactic factors; the former facilitating monocyte attachment to endothelium, and the latter resulting in migration of monocytes into the intima where differentiation of monocytes to macrophages occurs. Uptake of modified LDL by macrophages results in the formation of foam cells. Lipid-laden macrophages secrete a number of inflammatory cytokines and amplify inflammation in the arterial wall.

Many studies have pointed to a positive relation between plasma hsCRP and the risk of coronary heart disease. In a meta-analysis of a total of 1,053 coronary events from seven prospective studies, a relative risk of 1.7 (95% confidence interval [CI] 1.4–2.1) was demonstrated for the top versus bottom third of the baseline plasma hs-CRP, estimated to be 2.4 mg/L and 1.0 mg/L, respectively. Thus it might be considered that hs-CRP is an independent marker of the risk of cardiovascular disease.

2.4.2. Cancer

Chronic inflammation related to certain infections and other causes is linked to increased risk of certain cancers, and malignant cells themselves secrete pro inflammatory cytokines which in turn enhance progression. Nuclear factor-κB (NF-κB) seems to play a pivotal role in the inflammation-cancer link. Microbial pathogens and tissue necrosis lead to the activation of NF-κB and other transcription factors, and this activation up-regulates expression of pro-inflammatory cytokines, cyclooxygenase-2 enzymes and other molecules enhancing tumor growth and progression. It is thus natural to investigate the association between inflammatory markers and cancer risk. The association with hsCRP has most frequently been studied for colorectal cancer. In patients undergoing surgery, higher CRP levels were related to a higher risk of recurrence and of dying from colorectal cancer (Nanri et al, 2007).

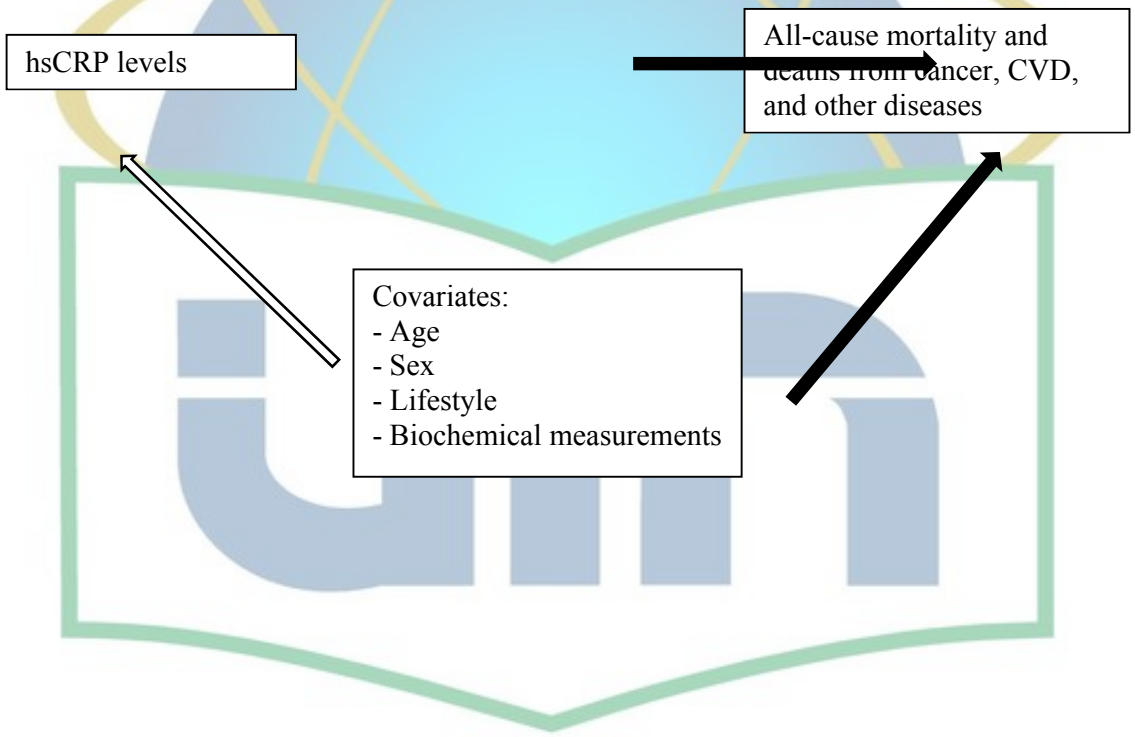
2.4.3. Cardiovascular disease

In January 2003, a joint panel of experts from the American Heart Association (AHA) and the Centers for Disease Control and Prevention (CDC) released a statement acknowledging that testing for CRP is useful in determining a patient's risk for cardiovascular disease. A growing body of scientific data links CRP with cardiovascular events. Studies indicate that patients with the highest levels of CRP have about twice the risk as those with the lowest levels.

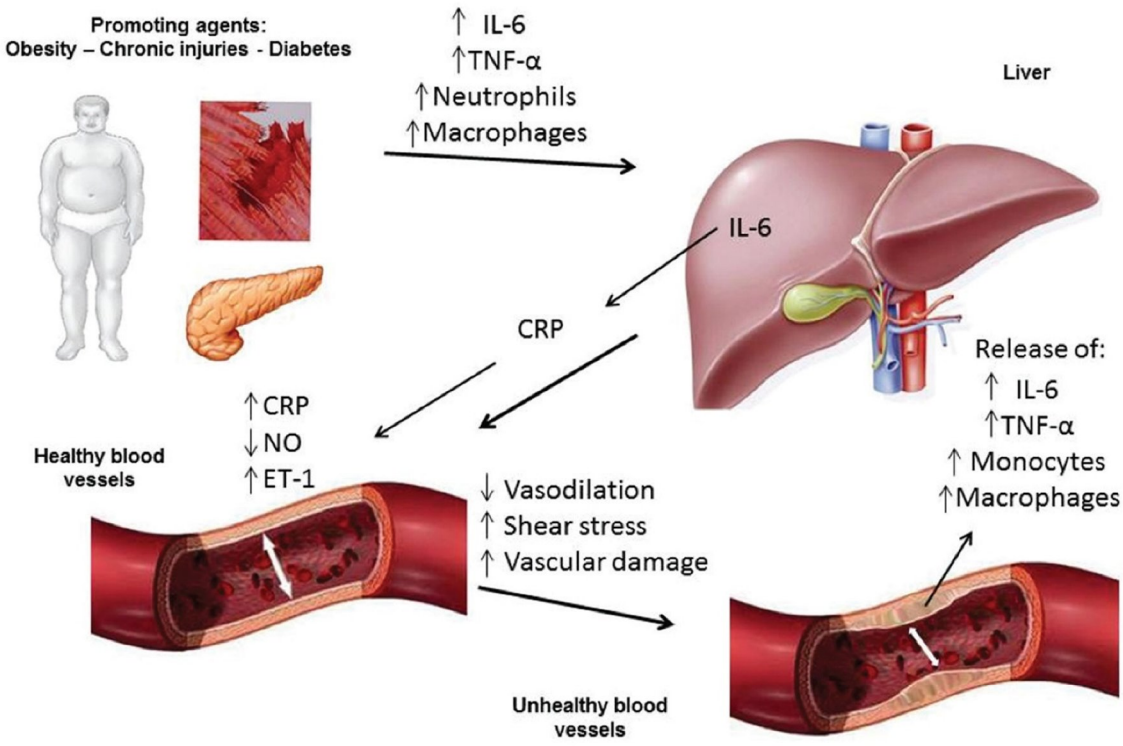
High plasma concentration of CRP was associated with a 2-fold increase in risk of stroke, a 3-fold increase in risk of myocardial infarction (MI), and a 4-fold increase in risk of developing peripheral vascular disease. Hypertension showed increased levels of several inflammatory markers, including soluble leukocyte adhesion molecules. Chemotactic and pro-inflammatory cytokines, specific growth factors, heat shock proteins and CD40L. CRP concentrations among hypertension might be explained by a clustering of common positive

CRP covariates (i.e. age, female sex, and increased body mass index and lipid concentrations) among hypertensive patients. The association between elevated CRP levels and high blood pressure may have three different pathophysiological explanations. The clinical relevance of CRP determination in subjects without clinically overt ischemic heart disease is still controversial. However, in a recent report of the Reykjavik Study, Danesh et al. confirmed CRP is an independent predictor of coronary heart disease, but the CRP-associated odds ratio was lower than that of established cardiovascular risk factor such as systolic blood pressure, smoking, and total cholesterol (Ingle et al., 2011).

2.4.4. Conceptual Framework



Etiology of the inflammatory process



CHAPTER III METHODS

3.1. HsCRP levels in Japanese subjects

3.1.1. Study subjects

Data were derived from the baseline survey of the Kyushu University Fukuoka Cohort Study on lifestyle-related diseases. The study was conducted with the approval of the Ethics Committee of Kyushu University Faculty of Medical Sciences, and written informed consent was obtained from all participants. Details of the survey have been described elsewhere (Nanri et al., 2008). The subjects were men and women aged 50-74 years living in the East Ward of Fukuoka City. A total of 12,948 subjects (5,817 men and 7,131 women) were recruited from February 2004 to August 2007, with participation rate of 24%. We excluded 1,862 subjects who had the following conditions: current medical care for cancer (*n*=498), ischemic heart disease (*n*=534), stroke (*n*=276), chronic liver disease (*n*=311), chronic renal failure (*n*=29), and alcohol addiction (*n*=4); prior history of ischemic heart disease (*n*=149) or stroke (*n*=158); and concentration of hsCRP >10 mg/L (*n*=212). Of the remaining 11,086 subjects, we further excluded 6 subjects with missing information on covariates. Our final sample for analyses included a total of 11,080 subjects (4,737 men and 6,343 women). To examine hsCRP levels in Indonesian subjects, we used data from hospitals.

3.1.2. Laboratory measurements

Recorded information was referred to regarding eight items of serum biochemistry including alanine aminotransferase (ALT), γ -glutamyltransferase (GGT), total cholesterol, and high-density lipoprotein (HDL)-cholesterol if these measurements had been done in the past year. When recorded information was not available, 5 mL of venous blood was taken for

the measurements, and serum samples frozen in dry ice were shipped to an external laboratory (SRL, Hachiohji, Japan).

Glycated hemoglobin (HbA 1c) and serum concentrations of hsCRP were measured with all the participants. HbA 1c was assayed by using a latex agglutination turbidimetry, and hs-CRP was assayed by using a latex-enhanced immunonephelometric assay on a BNII analyzer (Dade Behring, Marburg, Germany). Both HbA 1c and hs-CRP were measured at an external laboratory (SRL, Hachiohji, Japan). As for hs-CRP, the limit of detection was 0.05 mg/L, and a value of 0.025 mg/L was arbitrary assigned when the value was below the detection limit.

3.1.3. Follow-up and ascertainment of mortality

We followed up the participant's vital status of Japanese from the study entry to 31 December 2013. Information on death and causes of death was obtained from a record link with the national death certificate files in Japan. Main outcome of interest were all-cause mortality (defined as death from any cause) and mortality from cancers, CVD, and other diseases as underlying cause. Mortality from other diseases was death from diseases other than cancer and CVD. The cause of death was classified according to the International Classification of Diseases, 10th revision (ICD-10). Deaths were coded as C00-C97 for cancer and I00-I99 for CVD.

3.1.4. Baseline variables

Data on lifestyle measures were collected by self-administered questionnaires. The questionnaire inquired about smoking, alcohol consumption, physical activity, sleeping, stress, dietary intake, current or previous treatment of diseases, use of drugs and supplements, and family history of selected diseases. The returned questionnaire was confirmed by a nurse

or a physician, and missing or inconsistent answers were clarified by in-person interview. Each subject undertook blood pressure measurement, anthropometric measurements (height in cm, body weight in kg, waist in cm, and hip in cm), and venous blood drawing.

Smokers were defined as those who had ever smoked one or more cigarettes daily for one year or longer, and categorized into never smokers, past smokers, and current smokers. Alcohol drinkers were defined as those who had drunk alcoholic beverages at least once per week over the period of 1 year or longer. The total ethanol intake per day was estimated on the basis of beverage-specific ethanol concentrations. Alcohol drinkers were classified as lifelong non-drinkers, past drinkers, and current drinkers. Questions on physical activity ascertained work-related and leisure-time physical activities over the previous year. With consideration to intensity in terms of metabolic equivalent (MET) and amount of time for each physical activity, MET-hours were calculated for work-related and leisure-time physical activity separately. Body mass index (BMI; in kg/m^2) was calculated. We slightly modified a 43-item food-frequency questionnaire for dietary assessment (Tokudome et al., 2004) and added five alcohol beverages, six-non alcohol beverages, and eight specific food items. A total of 49 food items were used to analyze a prudent dietary pattern characterized by higher intake of vegetables, fruits, and whole grains. A prudent dietary pattern was inversely associated with hsCRP in men and women of the present population (Nanri et al., 2008), and was considered as a covariate in the present study. Blood pressures were measured twice in a sitting position using an automated digital device (HEM-707, OMRON, Kyoto), and the second reading was used for the present study. Hypertension was systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or regular antihypertensive medication. Type 2 diabetes mellitus was determined by HbA1c of ≥6.5% or any anti-diabetic medication. Subjects with non-HDL cholesterol ≥170mg/dL were defined as having elevated non-HDL cholesterol (Shimano et al., 2008).

3.1.5. Statistical analysis

We calculated a follow-up time for each subject starting from the date of interview until the date of death or 31 December 2013, whichever came first. All analyses were sex-specific to explore potential differences in shape and magnitude of the associations in men and women. Vital status of men and women were compared using χ^2 test and Wilcoxon Mann-Whitney tests for categorical and continuous variables, respectively. Log transformation was applied to hsCRP to normalize the distribution. The hsCRP was analyzed as both continuous and sex-specific tertiles (0.03–0.33, 0.34–0.84, and 0.85–9.91 mg/L for men and 0.03–0.25, 0.26–0.61, and 0.62–9.80 mg/L for women).

We used Cox proportional hazard regression models to calculate hazard ratios (HR) and 95% confidence interval (CI). The models were tested and plotted based on scaled Schoenfeld residuals to confirm that assumptions of proportional hazard by hsCRP tertiles were not violated. Multivariate models included adjustment for age (continuous), smoking (never, past, and current smoking), alcohol intake (never, past, and current drinking), BMI (<22.5, 22.5-24.9, and ≥25.0), hypertension, and type 2 diabetes mellitus. HR of mortality per 1-SD higher log hsCRP (SD=1.08) is equivalent to that for a threefold higher hsCRP on the original scale (mg/L). Trend of the association was assessed using the tertiles rank as a continuous variable in the regression models. Interactions between hsCRP tertiles and sex in the regression models was tested for by computing a likelihood ratio test comparing the statistical fit of models with and without a two-factor interaction term. A two-sided *p*-value <0.05 was considered as statistically significant. Statistical analyses were calculated using SAS version 9.2 (SAS Institute, Cary, NC).

3.2. HsCRP levels in Indonesian subjects

To examine hsCRP levels among Indonesian subjects, we used a study reported using medical records, conducted at Medistra Hospital Jakarta. Details of the methods as follows. The subjects of the study were patients with coronary heart disease, from stable angina pectoris, unstable angina pectoris, and acute myocardial infarct who are treated and hospitalized at Medistra Hospital, Jakarta.

The sampling was done consecutively. The inclusive criteria were as follows: (1) patients with coronary heart disease, including stable angina pectoris, unstable angina pectoris, and acute myocardial infarction; (2) patients with coronary heart disease who have undergone history taking, physical examination, basic laboratory assessment, cardiac enzyme assessment, high sensitivity reactive C-protein levels at arrival, coronary angiography, and left ventriculography. The exclusion criteria were as follows: (1) acute infection in the last 2 weeks; (2) tissue injury of surgical wound within the last 2 weeks; (3) rheumatoid arthritis or other autoimmune disease; (4) liver or renal dysfunction.

In this study, the criteria for stable angina pectoris was based on the Canadian Cardiovascular Society Classification System as follows: patients with known coronary arterial disease where regular daily activity does not causes angina. Unstable angina pectoris is in line with the Braunwald classification, of specific chest pain accompanied by one of the following clinical findings: (1) Angina at rest, usually of over 20 minutes in duration within the last week; (2) new onset angina of at least class III or IV in the CCSC classification system in the last 2 months; (3) angina that has aggravated since the time of diagnosis, becoming more often, having longer duration of episodes, or a reduced threshold. While the definition of acute myocardial infarction is based on the 1979 WHO diagnosis, which is when 2 of 3 of the following symptoms are found: (1) infarct-specific chest pain: longer (over 30 minutes) and more severe, usually not alleviated by nitrates, and often requiring

morphines/opioids; (2) Electrocardiographic abnormalities and specific evolution; (3) increased blood cardiac enzymes (CK, CK-MB, Troponin T/I) of at least twice the normal upper limit. Quantitative assessment of C-reactive protein level was performed using the enzymatic immunometric assay or chemiluminescence assay, which has a high sensitivity with a 97.5 percentile level of 11 mg/L and could detect inflammatory processes down to a minimum level of 0.01 mg/dL. The extent of coronary lesion is determined based on cardiac angiography and based on a clinical scoring, stated as single vessel disease, double-vessel disease, and three-vessel disease. Quantitative cardiac angio-graphy illustrates the extent of coronary lesion using a scoring system, as the number of blood vessels with significant stenosis (lumen diameter reduction of 70% or more). Scores range from 0 to 3. The score according to AHA/ACC is called the extent score, measuring the involvement of coronary blood vessel segments. Evaluation of cardiac systolic function is performed as an ejection fraction calculated from the left ventriculography and stated in percentages. The study for examining the hsCRP levels among Indonesian subjects did not encounter ethical problems, as samples were obtained from retrospective medical record data. The authors ensured secrecy of the medical record data in line with medical ethics.

CHAPTER IV RESULTS

4.1. HsCRP levels in Japanese subjects

Behavioral and clinical characteristics by geometric mean hsCRP concentration in men and women have been previously reported (Hirata et al., 2012). Briefly, smoking, BMI, hypertension, type 2 diabetes mellitus, and elevated non-HDL cholesterol were positively associated with hsCRP concentration in both men and women. Past smokers had slightly higher hsCRP concentration than non-smokers in men ($p<0.001$) and women ($p=0.03$). The associations of hsCRP concentration with work-related physical activities in women and leisure-time physical activities in men and women were not significant in the multivariate analysis. An inverse association between work-related physical activity and hsCRP concentration was observed in men ($p<0.001$). Men with a less than 30 mL/day intake of alcohol had the lowest hsCRP concentration, and the association of alcohol intake and hsCRP in men showed a reversed J-shape. There was no such association with alcohol intake in women. Statin use was not associated with hsCRP concentration in men and women.

A total of 436 deaths (292 men and 144 women) occurred among the 11,080 participants during a median follow-up of 8 years (range, 0.1-9.9 years). Over half (53%) of all deaths (N=436) was attributable to cancer, and CVD deaths accounted for 15% of all deaths. A higher proportion of those who died during follow-up was male (67%). Baseline characteristics of men and women by vital status are summarized in Table 1.

Table 1. Baseline characteristics of men and women according to vital status

Variable	Men, *n*=4,737			Women *n*=6,343		
	Alive, *n*=4,445	Deaths, *n*=292		Alive, *n*=6,199	Deaths, *n*=144	
	(%)	(%)	*p* value[a]	(%)	(%)	*p* value[a]
Age (year), mean±SD	62.2±6.7	66.6±6.1	$<10^{-4}$	61.8±6.9	66.9±6.2	$<10^{-4}$
Body mass index (kg/m^2)						
<22.5	34.0	41.4	0.01	52.7	45.8	0.11
22.5 – 24.9	37.8	29.8		28.1	28.5	
≥ 25.0	28.0	28.8		19.2	25.7	
Smoking (cigarettes/day)						
Never	26.4	18.1	0.003	88.6	87.5	0.15
Past	42.4	43.2		5.1	8.3	
Current	31.2	38.7		6.3	4.2	
Alcohol intake (mL/day)						
Never	21.3	19.5	0.001	70.5	75.0	0.50
Past	6.0	11.3		2.4	2.1	
Current	72.7	69.2		27.1	22.9	
Work-related activity (MET-hr/day)[b]						
Q1	30.6	30.5	0.79	21.6	29.8	0.02
Q2	13.7	11.6		31.3	35.4	
Q3	29.0	30.5		21.4	17.4	
Q4	26.7	27.4		25.7	17.4	
Leisure-time activity (MET-hr/week)[b]						
Q1	23.1	22.9	0.40	26.3	28.5	0.91
Q2	27.3	25.7		26.8	27.1	
Q3	24.8	22.3		21.5	19.4	
Q4	24.8	29.1		25.4	25.0	
Prudent dietary pattern (factor score)[b]						
Q1	39.6	36.0	0.44	13.8	7.64	0.02
Q2	26.3	26.0		23.8	21.5	
Q3	21.2	22.3		28.6	25.7	
Q4	12.9	15.7		33.8	45.1	
Hypertension	60.9	67.1	0.04	45.7	56.3	0.01
Type 2 diabetes mellitus	9.5	19.5	$<10^{-4}$	4.0	9.7	0.001
Elevated non-HDL cholesterol	26.2	25.0	0.64	34.7	31.9	0.49
Statin use	6.5	6.2	0.83	13.9	18.8	0.10
hsCRP (mg/L)[c]						
T1	35.9	24.3	$<10^{-4}$	34.5	28.5	0.001
T2	34.0	32.5		33.8	25.7	
T3	30.1	43.2		31.7	45.8	

[a]Difference between alive and death groups. [b]Quartile and [c]tertile categories.

Deceased men and women were older at baseline. In men, decedents were more likely to be past smoker and current drinker, and had a lower baseline BMI than men who were alive. Work-related physical activity and prudent dietary pattern were not different between the 2 groups in men. Deceased women were less likely to physically active at work and more likely to have prudent dietary pattern than women who were alive, but BMI, smoking, and alcohol intake were not different between alive and death group in women. Deceased men and women had higher proportions of hypertension and type 2 diabetes mellitus at baseline than survivors. Leisure-time physical activity, elevated non-HDL cholesterol, and statin use were not different between alive and death group in men and women, but hsCRP level was clearly different, with higher proportions of hsCRP 3rd tertile in those who died. Cumulative hazards (Figure 1) illustrates the higher hazards over time for all-cause death in men (p=0.0005) and women (p=0.06) with the higher hsCRP level. The proportionality assumptions of the hazard by hsCRP tertiles for these outcomes were satisfied.

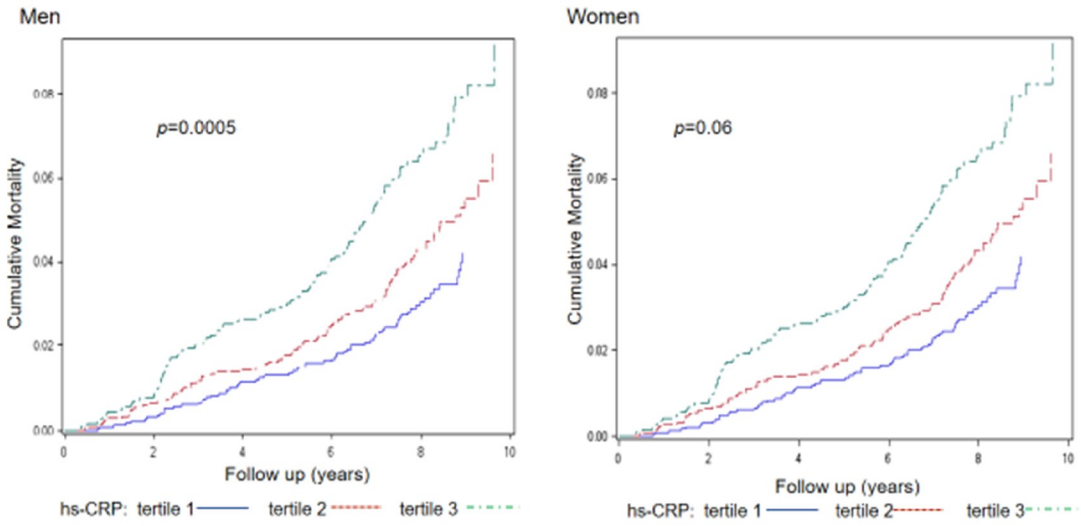


Figure 1. Cumulative hazards of all-cause mortality according to sex-specific hsCRP tertiles after adjustment for age, smoking habits, alcohol intake, body mass index, hypertension, and type 2 diabetes mellitus.

In the multivariate Cox proportional hazard regression model (Table 2), HRs for all-cause mortality were progressively increased with higher hsCRP tertiles, especially in men. Significantly increased HRs for all-cause mortality was found in men with hsCRP 3rd tertile. The results in men did not change with additional adjustment for work-related activity (sex-specific quintiles), prudent dietary pattern (quintiles), and statin use; HRs (95%CI) for the 2nd and the 3rd hsCRP tertiles were 1.27 (0.93-1.74) and 1.75 (1.30-2.36), respectively (*p* for trend=0.001). Although women with hsCRP 3rd tertile had a somewhat increased in risk of all-cause mortality, the increase was not statistically significant. Results derived using geometric mean hsCRP in associations with risk of all-cause mortality in men and women were consistent with the results derived from hsCRP tertiles.

Table 2. Adjusted hazard ratios for all-cause mortality in relation to hsCRP levels in men and women

hsCRP (mg/L)	N[a]	Age-adjusted HR (95% CI)	Multivariate HR[b] (95% CI)
Men (*n*=4,737)			
Continuous[c]	292/4,445	1.30 (1.17-1.44)	1.26 (1.13-1.40)
Tertile 1 (0.03-0.33)	71/1,597	1.00 (referent)	1.00 (referent)
Tertile 2 (0.34-0.84)	95/1,512	1.32 (0.97-1.79)	1.27 (0.93-1.73)
Tertile 3 (0.85-9.91)	126/1,336	1.91 (1.43-2.56)	1.75 (1.30-2.37)
	P for trend	$<10^{-4}$	0.001
Women (*n*=6,343)			
Continuous[c]	144/6,199	1.15 (0.98-1.34)	1.12 (0.95-1.32)
Tertile 1 (0.03-0.25)	41/2,139	1.00 (referent)	1.00 (referent)
Tertile 2 (0.26-0.61)	37/2,094	0.76 (0.49-1.18)	0.73 (0.47-1.15)
Tertile 3 (0.62-9.80)	66/1,966	1.28 (0.86-1.90)	1.19 (0.78-1.81)
	P for trend	0.04	0.07
P for interaction by gender		0.31	

[a]Number of deaths/alive. [b]Adjusted for age, smoking habits, alcohol intake, body mass index, hypertension, and type 2 diabetes mellitus. [c]Geometric means of hsCRP.

Cause-specific mortality analyses (Table 3) showed that hsCRP concentrations were positively associated with risks of deaths from cancer and CVD in men, and the HRs for mortality from other diseases in men were marginally significant. Mortality risks from cancer, CVD, and other diseases increased with 1-SD increment in the log hsCRP in women, but the HRs were not statistically significant. There were no statistically significant interactions between hsCRP and sex in all models of mortality risk.

Table 3. Adjusted hazard ratios for cause-specific mortality in relation to baseline serum hsCRP levels

	Cancer HR (95%CI)	CVD HR (95%CI)	Other diseases HR (95%CI)
Men (*n*=4,737)			
Age-adjusted	1.31 (1.14-1.51)	1.44 (1.08-1.92)	1.22 (1.01-1.47)
Multivariate-adjusted[b]	1.28 (1.11-1.48)	1.40 (1.40-1.88)	1.21 (1.00-1.47)
N[c]	162	38	92
Women (*n*=6,343)			
Age-adjusted	1.02 (0.81-1.28)	1.43 (1.01-2.03)	1.20 (0.92-1.58)
Multivariate-adjusted[b]	1.00 (0.79-1.26)	1.43 (0.99-2.05)	1.17 (0.88-1.56)
N[c]	69	28	47
P for interaction	0.15	0.23	0.54

[a]Geometric means of hsCRP. [b]Adjusted for age, smoking habits, alcohol intake, body mass index, hypertension, and type 2 diabetes mellitus. [c]Number of deaths.

4.2. HsCRP levels in Indonesian subjects

The subjects that participated in the study consisted of 106 coronary heart disease patients with the following characteristics distribution: most patients were male (87 patients, 82.1%), while only 19 were female (17.9%); the most common clinical indication was stable angina pectoris (90 respondents, 84.9%), followed by unstable angina pectoris in 11 patients

(10.4%) and acute myocardial infarct in only 5 patients (9%); the most common extent of coronary heart lesion among the respondents in this study was single vessel disease, found in 56 subjects (52.8%), even though the frequency was not much different with those with double-vessel disease (41 subject, 38.7%), with only 9 subjects with three-vessel disease (8.5%). Based on the risk factor, 76 subjects (71.7%) were found with hypercholesterolemia. Other quite dominant risk factors included hypertension, found in 60 subjects (56.6%), and acute myocardial infarction in 58 subjects (54.7%). From the subjects included in this study, there were almost as many patients for whom smoking was a risk factor compared to those for whom it was not (51 and 55 patients – 48.1% and 51.9% respectively).

The mean respondent age in this study was 58 years, with the youngest being 34 years and the oldest 82 years of age. The mean high sensitivity C-reactive protein in this study was 5.9 mg/L ± 14.1, with a minimum level of 0.2 mg/L and a highest level of 132.0 mg/L. Based on sex, the mean CRP level for males was higher than the mean CRP level for females, 13.2 mg/L and 4.4 mg/L respectively. The mean ejection fraction was 65.4%, with a minimum EF of 29% and a maximum of 80% (Table 4).

Table 4. Distribution of characteristics and hsCRP levels in Indonesian subjects

Subject Characteristic	N (%)	Mean ± SD	Median	Minimum Value	Maximum Value
Sex					
Male	87 (82.1)				
Female	19 (17.9)				
Extent of coronary lesion					
Single Vessel Disease	56 (52.8)				
Double Vessel Disease	41 (38.7)				
Three Vessels Disease	9 (8.5)				
Clinical indication					
Stable Angina Pectoris	90 (84.9)				
Unstable Angina Pectoris	11 (10.4)				
Acute myocardial infarction	5 (4.7)				
Risk factor					
Smoking	51 (48.1)				
Diabetes Mellitus	35 (33)				
Hypertension	60 (56.6)				
Hypercholesterolemia	76 (71.7)				
Hypertriglyceridemia	21 (19.8)				
Old myocardial infarction	58 (54.7)				
Post PTCA	35 (33)				
Post CABG	7 (6.6)				
Age		58.7± 10.6	57	34	82
Hs-CRP (mg/L)		5.9 ± 14.1	2.5	0.2	132.0
hs-CRP (mg/L) level					
- Male		13.2 ± 29.8		0.5	132.0
- Female		4.4 ± 6.6		0.2	36.5
Ejection Fraction (%)		65.4 ± 12.4	70	29	80

CHAPTER V DISCUSSIONS

5.1 HsCRP levels in Japanese subjects

In the large Japanese community-based study, elevated hsCRP levels were associated with the increased risk of all-cause mortality as well as deaths from cancer and CVD in apparently healthy Japanese men, but not women. Some studies have addressed the association between CRP level and mortality risk by sex (Iso et al., 2009; Ko et al., 2012; Ahmadi-Abhari et al., 2013; Doran et al., 2013; Sung et al., 2014; Wulaningsih et al., 2016).The present findings are consistent with the results from previous studies showing no association of hsCRP levels with all-cause (Ko et al., 2012; Sung et al., 2014), cancer (Ko et al., 2012; Wulaningsih et al., 2016), or CVD (Ahmadi-Abhari et al., 2013; Doran et al., 2013; Sung et al., 2014) mortality in women.

Although different cut-off hsCRP levels were used, two prospective studies showed that hsCRP levels were associated with the increased risks of all-cause and CVD mortality in men (Doran et al., 2013; Sung et al., 2014). For example, CRP level of ≥3 mg/L was associated with an increased risk of all-cause mortality in men but not women participated in the National Health and Nutrition Examination Survey (NHANES) III study (Doran et al., 2013). In addition, a strong and consistent dose-response relationship between CRP levels and CVD mortality was observed in men, while CVD mortality in women were statistically significant only at CRP levels of 5.0, 8.0, and 9.0 mg/L in that study (Doran et al., 2013). Although a higher hsCRP quartile was associated with an increased risk of CVD mortality in Japanese men and women during 13-year follow-up, the CVD mortality risk was higher in men than women (Iso et al., 2009). On the other hand, a recent analysis within the European Prospective Investigation of Cancer (EPIC)-Norfolk study showed that CRP levels of 3-10 mg/L was associated with increased risks of all-cause and CVD mortality in both men and

women (Ahmadi-Abhari et al., 2013). These findings suggest that inconsistent results between men and women are derived from differences in definition of elevated and clinical cutoff hsCRP.

Likewise, sex differences have been reported in the association between hsCRP level and cancer mortality. In the British women population, the increased risks of cancer mortality associated with elevated CRP concentrations were found in those who had or never had cancer at baseline, but the associations were not statistically significant after adjustment for IL-6 (Heikkila et al., 2007). In contrast with the results of the present study, the EPIC-Norfolk study showed that women had a small increase in the risk of cancer mortality associated with 1 mg/L increase in CRP after 17 year follow-up, but an association between CRP levels and cancer mortality was not found in men (Ahmadi-Abhari et al., 2013). Geometric means of CRP concentrations were higher in women than men in this study (Ahmadi-Abhari et al., 2013).

In the present study, the median of hsCRP was higher in men than in women, at 0.49 mg/L and 0.38 mg/L, respectively. In addition, the proportions of men (7.2%) and women (4.3%) with hsCRP concentrations ≥ 3mg/L are very low in the present study, indicating that distribution of hsCRP concentration is clearly low in Japanese. Unlike findings from the present study and other Asian studies (Saito et al., 2007; Nanri et al., 2011; Sung et al., 2014), several studies among Western population found that CRP concentration was higher in women than in men(Ahmadi-Abhari et al., 2013; Doran et al., 2013). The reason for the discrepancy in CRP levels with respect to sex is not clearly resolved, but genetic diversity has been reported to influence CRP levels (MacGregor et al., 2004). Furthermore, differences in lifestyle and metabolic risk factors between men and women have been suggested to affect CRP concentrations (Lee et al., 2009).

It remains unclear why elevated hsCRP is more highly associated with mortality risk in men, but not women. Higher hsCRP level may represent an ongoing inflammatory that impairs survival which warrant further investigation. In the present study, higher levels of hsCRP in men than women could be the reason of a greater number of deaths in men than women, which also reported in other studies of Asian subjects (Ko et al., 2012; Sung et al., 2014). In Western populations, women had higher CRP level at baseline than men, and not be at higher risk of mortality (Ahmadi-Abhari et al., 2013; Doran et al., 2013). A protective effect of endogenous female hormones may play a role in attenuating the effects of hsCRP (Gaskins et al., 2012). However, interpretation of the non-significant associations of hsCRP with mortality risks in women is rather difficult, particularly because sex hormone therapy is not commonly used (1.4%) among women in the present study. Another possible reason is that the effect of hsCRP is not the absolute value, but a somewhat increase in baseline hsCRP was associated with mortality (Parrinello et al., 2015).

The large size of the study population, sex-specific analysis, and control for important confounding factors were strengths of the present study. Several limitations need to be discussed, however. The hsCRP was measured only once which may have influenced the results due to within-person fluctuations, and thus tend to underestimate any associations. We excluded those subjects with life-limiting morbid conditions or acute inflammatory conditions to eliminate a random misclassification bias. Another limitation is that the self-reported on information of drug use, especially statin and HRT. However, this medication was rarely used in Japan, and this suggests that such a bias did not invalidate the present findings.

5.2. HsCRP levels in Indonesian subjects

One of the aims of this study is to determine the mean high sensitivity C-reactive protein level in patients with coronary heart disease, which amounts to 5.9 mg/L ± 14.1, with a median of 2,5 mg/L, and a lowest distribution of 0.2 mg/L and a highest value of 132.0 mg/L. After univariate statistical analysis using a computer program, the frequency distribution of the levels was found to be abnormal, so the first choice for analysis is by logarithmically transforming the CRP value (log natural). However, if this method is used, there will be difficulty in conducting clinical interpretation. Another analytical alternative is to use the non-parametric statistics. Bivariate analysis between the mean C-reactive protein level and extent of coronary lesion was performed using the Kruskal Wallis test, with a result of p=0.056. This means that that statistically, the mean C-reactive protein level and extent of coronary lesion portrayed as single-vessel disease, double-vessel disease, and three-vessel disease does not show a significant correlation. The results is not far from previous studies, seen from the mean CRP levels for each group of lesion extent, as follows: 5.5 mg/L in single vessel disease (SVD), 6.6 mg/L in double-vessel disease (DVD), and 5.5 mg/L in triple-vessel disease (TVD).12 The lower CRP level in TVD lesions compared to DVD lesions may be due to the fact that CRP illustrates an ongoing inflammatory process, while coronary angiography demonstrates stenosis in coronary blood vessels. The lesion may be minimal (such as a less than 70% stenosis of only one blood vessel), but if the CRP level is high, references state that there is a greater risk of cardiovascular event such as acute myocardial infarct or sudden death due to cardiac causes compared to in patients without increased CRP levels. Based on the results of the analysis of mean CRP level for each clinical condition, the mean CRP level among patients with stable angina pectoris was 5.4 mg/L, a higher 9.3 mg/L in unstable angina pectoris, and then reduced again at 8.2 mg/L for acute myocardial infarct. Even though the differences in these mean averages were not significant for each clinical indication, with such mean averages, we must continue to be alert for the possibility.

For the mean CRP levels obtained in this study, the interpretation should be in line with the method and the kit used by the laboratory to examine the C-reactive protein. The kit stated <11 mg/L as the normal level, while several studies state the normal level as 3.0 mg/L. Another problem that occurs in the interpretation of CRP results is the lack of a cutoff point, or percentile, quartile, quantile, and other terms as used in several references, for the Indonesian population. The values based on foreign references cannot be used completely, since there needs to be a large scale study in Indonesia to determine the normal range for Indonesians. In this study, the normal level used was 11 mg/L, and seeing the mean C-reactive protein level of 5.9 mg/L, there were not many subjects with a high range of C-reactive protein level, but there is a tendency for it to increase to 5.4 mg/L in stable angina pectoris, 9.3 mg/L in unstable angina pectoris, and 8.2 mg/L in acute myocardial infarct. Sano et al found that among patients within 6 hours of acute myocardial infarction that underwent CK-MB, troponine T, and C-reactive protein, the data demonstrates no difference in CK-MB and troponine T levels during admission, while C-reactive protein levels are already elevated. This demonstrates that CRP levels demonstrate previous coronary lesion prior to plaque rupture, and does not demonstrate myocardial necrosis. Plaque rupture usually occurs in areas where the fibrous cap is thin and infiltrated by macrophages.

CHAPTER VI CONCLUSIONS

6.1. HsCRP levels in Japanese subjects

1. In the large Japanese community-based study, baseline hsCRP was an independent predictor of all-cause mortality as well as deaths from cancer and CVD in apparently healthy Japanese men, but not women.
2. The present findings suggest that hsCRP may have a different effect on the risk of death in men and women, thus warrant further investigation.

6.2. HsCRP levels in Indonesian subjects

1. The male patients in this study had a higher mean C-reactive protein level compared to the females.
2. The mean C-reactive protein level in the population of the study respondents was not too high compared to the level determined by the laboratory assessing the C-reactive protein level.

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FIGURES

TABLES