

Study of Novel Markers for Early Diagnosis of Cardiovascular Diseases*

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ABSTRACT

In our country, cardiovascular disease (CVD) and Coronary heart diseases (CHD) are the leading causes of death. It is well known that CHD is multifactorial, involving environmental factors such as diet, level of exercise and cigarette smoking, and inherited factors. According to the statistical data in 2003, the cause of death with the highest mortality was including hypertension, ischemic heart disease and atherosclerosis, which accounted for 24.7% of total mortality. In spite of, there have been few study reports on the change of biochemical markers and mechanisms concerned. The development of biochemical markers is required for an early diagnosis and treatment of cardiovascular diseases that are related with dietary habits of Korean people enjoying mixtures of traditional dietary style and westernized food-styles. Therefore, the most efficient cost-saving biochemical marker was established in this study, through analysis of biochemical markers related with dietary habits which are susceptibly being changed in association to cardiovascular diseases from the pre-disease phase, and through reanalysis and assessment of early diagnosis of and preventive effects of diagnosis of cardiovascular diseases by demographical character including sex, age, and socioeconomic level with use of biochemical markers that are identified and selected among the parameters in consideration of the effectiveness and appropriateness of early diagnosis of diseases. The appropriateness of biochemical markers was reviewed by professionals (medical, pharmaceutical area and food/ nutrition area) and CRP (C-Reactive Protein) was identified to be possible in Korea. It is thought that these biochemical markers may be used as the basic data for early diagnosis and prevention of cardiovascular diseases (CVD) which may be used for Korean people. (*J Community Nutrition* 6(3) : 155~163, 2004)

KEY WORDS : cardiovascular disease (CVD) · biochemical marker · C-reactive protein.

Introduction

Cardiovascular diseases (CVD) and coronary heart diseases (CHD) are the leading causes of death and disabilities and are increasing rapidly in the western world (WHO 2004). By the year 2020, it is estimated that CVD and CHD will surpass infectious diseases as the world's leading causes of death and disabilities (Murray et al. 1997). Cardiovascular diseases are the ones that are caused by shrinkage or occlusion of coronary arteries with arteriosclerosis after inflammation and thrombus of coronary artery of the heart, and the factors participated in

this mechanism may possibly be used in diagnosis of coronary heart diseases and their follow up monitor. The major modifiable risk factors include cigarette smoking, obesity, elevated total and low density lipoprotein cholesterol (LDL-c) levels, and diabetes mellitus (U.S. Preventive Service Task Force 2001). A substantial body of evidence now supports reducing these factors to reduce morbidity and mortality associated atherosclerotic vascular disease. In recent years, a number of candidate risk factors or markers have been proposed as significant predictors of arteriosclerosis and its complications (Table 1). This review will highlights biochemical markers which are used in hospitals and 4 important emerging new risk predictors and other risk factors which are related to CHD : C-reactive protein (CRP), lipoprotein (a) [Lp(a)], fibrinogen, homocysteine and urine albumin creatinine ratio (UACR), serum amyloid A (SAA) etc. The 4 risk predictors were selected because there is substantial evidence on their predictive abilities, there is a genetic basis for premature

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Table 1. Biochemical markers of CVD and CHD

Biochemical markers	
Inflammatory markers	C-reactive protein, interleukins, serum amyloid A (SAA), vascular and cellular adhesion molecules Soluble intercellular adhesion molecule (sICAM-1)
Hemostasis/thrombosis markers	Fibrinogen, plasminogen activation inhibitor 1 (PAI-1)
Lipid related factors	Small dense low-density lipoprotein (LDL) Lipoprotein (a) Oxidized LDL
Other factors	Homocystein, lipoprotein-associated phospholipase A (2) Urine albumin creatinine ratio (UACR) APOC3 (apolipoprotein C-III), CEPT (cholesterol ester transfer protein) Cardiac troponin (cTn), creatine kinase (CK), CK-MB (creatine kinase-subunit of M and B of creatine kinase)

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disease, modifying treatments are available, and/or these factors are the subject of ongoing or completed clinical trials (Table 2).

Methods

Using the terms atherosclerosis, cardiovascular disease, risk factors, prevention, screening, C-reactive protein, lipoprotein (a), fibrinogen, homocysteine, SAA, and UACR etc, we searched the MEDLINE database from January 1995 to January 2004. Conference proceedings, abstract booklets, and bibliographies of pertinent articles and books were hand searched to identify additional articles. We selected original investigations and reviews of the epidemiology of atherosclerosis and the associations of conventional and novel risk factors with vascular risk. A diverse array of studies were examined, including controlled trials, prospective cohort studies, systematic overviews, case control, cross-sectional, and mechanistic studies. We focused our attention on assessing the clinical significance and additive predictive value of candidate risk factors in comparison with established and validated risk prediction tools, such as the Korean Medical Insurance Corporation (KMIC).

Results

1. Candidate biochemical markers of CVD and CHD

Especially, numerous risk factors for CVD have been identified, many of which are modifiable by western diet and lifestyle practices. Major modifiable risk factors include cigarette smoking, elevated total and low density lipoprotein cholesterol (LDL-c) levels, obesity, hypertension, diabetes

mellitus, and a sedentary life style (U.S. Preventive Service Task Force 2001). Other important risk factors that are modifiable by diet are a high level of triglycerides (TG), lipoprotein (a), insulin, hypertension, altered hemostatic factors, and small dense LDL particles (Cullen 2000).

It has been proven that each of total cholesterol (TC), LDL-c, high density lipoprotein cholesterol (HDL-c), and TG is an independent risk factor of coronary artery disease (Kim et al. 2001).

In determination prevalence rate of risk of cardiovascular diseases, the susceptibility was 91.2% when either of LDL or HDL-c is not lower than standard, and 73.9% when LDL/HDL-c ratio was settled not lower than 3.5 (Wilson et al. 1998).

Although LDL-c may be calculated with use of Friedewald equation ($[LDL-c] = [TC] - [HDL-c] - [TG]/5$) (NIH 1995), the result is not accurate when TG level is 400mg/dl or higher, and the direct determination method is currently preferred. According to Framingham Research, relative risk of TC to cause CVD in men was 1.31 (95% confidential interval, 1.01 – 1.68) in 200 – 239mg/dl in comparison to 200mg/dl or lower level, and 1.90 (95% confidential interval, 1.47 – 2.47) in 240mg/dl or higher level; attributable risk was 10% and 17%, respectively, and the relative rate was as higher as 1.68times (95% confidence interval, 1.22 – 2.29) with high TG and lower HDL-c values. In a Korean Medical Insurance Corporation (KMIC) study that was carried out with Korean subjects that consisted of public service personnel and school personnel, the relative risk was 11% (95% confidence interval, 5.9 – 12.5) and 8.9% (95% confidence interval, 6.6 – 14.0) (Kim et al. 2001).

Oxidative damage to the endothelium may be important in

Table 2. New candidate biomarkers of CVD and CHD in Korea

Risk factor	Biological function	Increase factor	Decrease factor
C-reactive protein (CRP)	Acute phase reactant	Inflammation Smoking HRT (estrogen and progesterone)	Weight loss Physical activity
Lipoprotein (a)	Acute phase reactant	Inflammation Genetics	Nutrient - Niacin HRT (estrogen and progesterone)
Fibrinogen	Acute phase reactant	Inflammation Smoking Age	Smoking cessation Fibrates Nutrient-niacin exercise
Homocysteine		Nutrient- Folate/B6/B12 deficiency Genetics	Nutrient- Folate/B6/B12 replacement Genetics

HRT : hormone replacement therapy, LDL : low density protein
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initiation and progression of atherosclerosis. And it was known that Serum β -carotene and α -tocopherol concentrations are low in the persons with hyperlipidemia and that intake of antioxidant vitamin C and E is low in myocardial infarction patients (Baiqiang et al. 2003). It was reported that malondialdehyde (MDA) and thiobarbitric acid reactant substance (TBARS) are increased with oxidation of LDL and are improved with administration of antioxidant vitamins. In recent years, a number of new candidate risk factors or markers have been proposed as significant predictors of atherosclerosis and its complications. Other risk predictors, SAA, and IL-6 (Verma et al. 2002) increased in patients with unstable angina pectoris and are also related to the frequency of incidence of complicated diseases during future attacks of coronary events and the period of hospitalization.

2. New candidate markers of CVD and CHD

1) CRP

At present, inflammation has recently been understood to be a major factor of occurrence of CVD and to participate not only in initiation of atherosclerosis process, but also in development to blood clotting rupture (Weintraub, Harrison 2000 ; Hoffmeister et al. 2001 ; Nader et al. 2002). Localized inflammation process was known to act as the role of a trigger towards CVD by unstable blood clotting. Several, but not all, prospective studies have reported a relationship between elevated concentrations of inflammatory markers and incident of clinical cardiovascular events (Wilson et al. 1998 ; Torzewski et al. 1998 ; Muir et al. 1999 ; Biasucci et al. 1999 ; Tommasi et al. 1999 ; Heeschen et al. 2000 ; Van et al. 2002 ; Danesh et al. 2002).

As these markers, CRP has been known to possess the

highest predictive capability among known markers of arteriosclerosis and vessel damage (Van et al. 2001). CRP is one of serum proteins that are significantly increased with diseases such as inflammation or tissue necrosis ; which is synthesized in the liver and its release is stimulated by interleukin 6 (IL-6 Paul 2000) and exists in normal human serum as a very small amount, and it participates in association of cardiovascular diseases through facilitation of intimal smooth muscle proliferation and reactive oxygen species (ROS) formation (Muir et al. 1999 ; Biasucci et al. 1999 ; Di Napoli et al. 2001). CRP is consistent to moderately strong markers of CVD, that is, the role of infection in pathogenesis of CVD in middle aged adults of Cohort study (Torzewski et al. 1998 ; Tommasi 1999 ; Heeschen 2000).

In Meta analysis of fourteen prospective long-term studies, Dansh et al. reported that the risk ratio of higher tertile to cause CVD in CRP level, in comparison to lower 1/3, was 1.9 (1.5 - 2.3, 95%) (8). Reviewing the results of various studies, CRP may be the predictive indicators of recurrence and death caused by ischemic heart disease (Muir et al. 1999 ; Biasucci et al. 1999 ; Di et al. 2001) acute atherosclerosis (Tommasi 1999 ; Heeschen 2000 ; Torzewski et al. 1998) and peripheral vessel disease. Therefore, CRP is a useful marker of the risk of CVD in not only the patients with CVD but also healthy persons with no disease. Although it is not apparent whether CRP is the marker or transmission factor of infection, there is evidence that CRP may take the direct role to arteriosclerosis. However, this relation is not CRP specific because other infection factors may explain this. Relative ratio (RR) was 2.6 when CRP was not 10 or more. CRP is increased by 90% in patients with unstable angina pectoris and by 13% in stable angina pectoris, thus, may be used in

diagnosis and prognosis of acute coronary artery symptoms. There was one study on predictive value, which was carried out in addition to established prediction models such as Framingham Risk Score (FRS), however, in this report, correction with body mass indices (BMI), abdominal fat, and physical activity was not made and blood pressure, diabetes, and smoking could not be considered accurate because they were based on self-reporting. Although retrospective studies showed that CRP screening is concurrent with use of statin class medicines may reduce the incidence of cardiovascular diseases even in the case of no hyperlipidemia; there has been no prospective clinical study (Torzewski 1998; Torzewski et al. 2000; Pasceri et al. 2000; Zwaka 2003). However, the use of CRP as the tool for global risk assessment of cardiovascular diseases is limited (Kushner, Sehgal 2002). First of all, CRP shows low specificity in the presence of chronic lung disease or inflammation and data on the population groups other than the white race are not sufficient (Ridker 1999; Paul et al. 2000; Peter et al. 2001). Also, it has a strong relation with risk factors (such as fibrinogen etc) of other cardiovascular diseases (Peter 2001; Tanne 2001; Danesh 2002; Nader et al. 2002).

2) Lipoprotein(a) [Lp(a)]

Lp(a) is considered to suppress secretion of tissue-plasminogen activator in endothelial cell and to cause atherosclerosis through transportation of cholesterol to damaged vessel area (Ridker 1999; Peter et al. 2001; Nader et al. 2002; Hong et al. 2003; Frederick et al. 2003). If Lp(a) is high in the situation of increased LDL-c, diabetes, decreased HDL-c, hypertension, hyperhomocysteinemia, and increased fibrinogen concentration, it may be more harmful (Foody 2000; Von Eckardstein et al. 2001; Solfrizzi 2002; Cantin 2002). Important is that there is no world-wide identified and standardized method to measure Lp(a) and lowering of Lp(a) is practically impossible, thus, study on reduction of Lp(a) and its relationship with cardiovascular diseases is difficult to be carried out. In Meta analysis of recent twenty seven prospective studies, the risk ratio of higher tertiary of Lp(a) was reported to be 1.6 (1.4 – 1.8, 95%) in comparison to lower tertiary. In addition, in case of increased LDL-c, DM, decreased HDL, hypertension, hyperhomocysteinemia, or increased fibrinogen level, the higher Lp(a) was, the higher the risk could be (Cleophas et al. 2000; Von 2001; Van et al. 2002; Solfrizzi 2002; Cantin 2002).

3) Homocysteine

Homocysteine was known to be independent from CVD and death associated to CHD was found to be decreased by decreasing homocysteine through administration of folic acid and vitamins (Biasucci et al. 1999; Christen et al. 2000; Ford et al. 2002). Homocysteine level was increased in case of congenital metabolic disorder of enzymes participating in homocysteine metabolism, and 50% of such patients shows arteriovenous thrombus by age 30 (Klerk et al. 2002; Wald 2002). It was found that the level of homocysteine is elevated and vascular diseases exist when 5,10-MTHFR participating in homocysteine metabolism is mutated (from 677C to T) (Klerk et al. 2002; Wald 2002). Two Meta analysis showed that mutant allele (TT) homozygote and odd ratio were 1.21 (1.06 – 1.39) and 1.16 (1.05 – 1.28), respectively, in comparison to wild-type homozygote (CC) (Klerk et al. 2002; Wald 2002).

Besides, many studies showed the correlation between homocysteine level and vascular diseases in general population and the patients with vascular diseases (Cleophas et al. 2000; Moller et al. 2000; Kelly et al. 2002; Bautista et al. 2002).

However, we found only one study of the risk prediction capabilities of homocysteine measurement with reference to the FRS (Framingham Risk Score). This study focused on a high-risk population with existing CVD and only overall mortality (and not cause-specific death). Also, it is not clear that homocysteine is the cause or the result of atherosclerosis, and to know whether it is the marker of currently existing vascular diseases; the result of current randomized controlled study should be confirmed, which is carried out with use of vitamins focused on decrease of homocysteine level (Daniel et al. 2003). Clinical trials carried out on the subjects with post coronary angioplasty showed that folic acid, vitamin B6, and vitamin B12 decreased the rate of restenosis and homocysteine levels. However, there was no reduction in myocardial infarction or death (Cleophas et al. 2000; Rolsom 1999; Solfrizzi et al. 2002).

4) Fibrinogen

Studies in healthy individuals have shown that plasma fibrinogen is a strong predictor for cardiovascular disease and mortality (Tanne et al. 2001). CHD patients were reported to have higher fibrinogen levels than healthy individuals. Fibrinogen is a circulating glycoprotein and is known to execute coagulation action at final stage of coagulation reaction when

vessels or tissues are damaged, to control cell adhesion, to contract damaged site of blood vessel, to facilitate platelet aggregation, to settle blood viscosity (Fey, Fuller 1987 ; Fowkes et al. 1995 ; Tanne et al. 2001) . In two Meta analysis of eighteen and twenty-two prospective long-term studies, the risk ratio and odds ratio of higher tertiary of fibrinogen were 1.8 (1.6 – 2.0) and 1.99 (1.85 – 2.13), respectively, in comparison to lower tertiary (Maresca et al. 1999 ; Moller et al. 2000) . In another meta-analysis of six prospective studies, the odds of sustaining a cardiovascular event in healthy persons with a fibrinogen level in the highest tertile were 2.3 times as high as in those with fibrinogen levels in the lowest tertile. Other studies reported that fibrinogen is related to ischemic stroke (Fey, Fuller 1987 ; Folsom et al. 1999 ; Tanne et al. 2001) , peripheral vascular diseases (Fowkes 1995) . Also, fibrinogen level has positive correlation with smoking quantity (Meade et al. 1987 ; Wilkes et al. 1988) , DM, hypertension, obesity, and sedentary life style (Meade et al. 1987 ; Maresca et al. 1999) . However, in a randomized controlled study on decrease of fibrinogen with use of bezafibrate to patients with peripheral vascular diseases, fibrinogen was decreased by 13% but incidence of coronary arterial diseases or cerebrovascular diseases was not decreased (Meade et al. 1987 ; Wilkes 1998) . Thus, it is required to carry out a study with the subject “Fibrinogen is the cause or the result of atherothrombosis?” In addition, there is only one study carried out focused on the additional usefulness of Framingham Risk Score (FRS) (Acevedo et al. 2002) .

5) Others

Urine Albumin Creatinine Ratio (UACR) was a known to marker of complicated cardiovascular disease in patients with hypertension and diabetes (Agewall et al. 1997 ; Jager et al. 1999 ; Jensen et al. 2000) . It was found that increased serum uric acid is an independent predictive parameter of cerebrovascular disease (Alderman et al. 1999 ; Weir et al. 2003) . However, use of UACR is limited as a parameter with unapparent absolute standard by which UACR increases the risk of cardiovascular disease as far as studies until now, and the studies are needed on general population in our country. Serum amyloid A (SAA) exists as apolipoprotein of HDL fraction with increase in inflammatory condition and is manifested with myocardial infraction. Soluble intercellular adhesion molecule (sICAM-1) is considered to be the parameter of future occurrence of acute myocardial infraction. Recently, the method

to measure APOC3 (apolipoprotein C-III) and CEPT (cholesterol ester transfer protein) has been developed.

Discussion

As reviewed above, no suitability for use in screening test in public health practice has been identified with various candidate risk factors as well as current biochemical marker for screening test of cardiovascular disease. Among the conditions for inclusion of CRP, fibrinogen, homocysteine, and Lp(a), which have relatively good research background for inclusion in routine screening, the problems to be solved are as follows : the first is the requirement for available standardized and accurate laboratory test ; Lp(a) does not satisfy this standard. The second is the requirement for supply with additional information to current approach method ; CRP, fibrinogen, and homocysteine are supported by only one study and Lp(a) is not supported by any studies. The third is the requirement for 1) additional support to current traditional targeting therapy (e.g., diabetes, hypertension, and hyperlipidemia etc.) from treatment of elevated levels or 2) the study to get the result of decreased cardiovascular end points by treatment of elevated levels of homocysteine. The fourth is difficulty in establishment of standards to be commonly applied to all the subgroups because diagnostic profile obtained through a certain screening test may be applied to the exact population group only. Accordingly, although CRP is the most adequate test method among the predictive parameters of CVD that are newly reviewed. But further study on the standard for prediction of occurrence of CVD with common population of the country as the subjects is still needed and cost-effect analysis is required for utilization of this result in screening of Koreans. Additionally, it is required to carry out large-scale long-term study to improve cardiovascular end points 1) to carry out test on measurement of UACR (urine albumin creatinine ratio) 2) serum uric acid levels and risk of cardiovascular disease occurrence 3) an effort should be made to correct traditional risk factors at the standpoint of health management. Only creatine kinase (CK), CK-MB (Creatine kinase -subunit of M and B of creatine kinase), and Cardiac Troponin (cTn) are used in assessment of susceptibility, specificity, and predictability, in diagnosis of cardiovascular diseases, and in follow-up observation of treatment, as biochemical markers and other tests such as EKG are more useful (Kim et al. 2002) . As CK and CK-MB, enzymes existing in myocar-

dium, not only shows high specificity, but also is increased in blood upon occurrence of myocardial infarction, they are most commonly used in diagnosis of CHD. However, they are frequently not useful in diagnosis of patients who do not visit hospitals. CK-MB changes in concentrations of tissue damage, and catalyzes the synthesis of phosphocreatine from creatine. CK activation is significantly high in patients with progressive amyotrophy or acute myocardial infarction and it is useful in diagnosis of myocardial infarction (Park et al. 1997 ; Russell et al. 2003).

Cardiac Troponin, a biochemical marker of myocardial infarction, which is recently most commonly used, is a protein controlling contraction of myocardium and is not found in normal blood ; with its cardiac-specificity, it is used as a more susceptible diagnosis marker of myocardial infarction in comparison to CK-MB. Concurrent use of Cardiac Troponin and CK-MB is useful in diagnosis of myocardial infarction recurrence, assessment of myocardial infarction treatment, and diagnosis of post-infarction angina pectoris. Atherosclerosis, the cause of cardiovascular diseases, is an inflammation reaction and a process of coagulation-dissociation ; the factors participating in this process may highly used in diagnosis of cardiovascular disease, follow-up observation of treatment and CRP and homocysteine are under identification. C-Reactive Protein is a useful marker of the risk of cardiovascular diseases in patients with cardiovascular disease and in healthy persons without cardiovascular disease. CRP with elevated susceptibility was strongly related with increase of risk of cardiovascular disease as well as fibrinogen is known to be related with myocardial infarction and spontaneous occurrence of ischemia. In summary, CK-MB and Cardiac Troponin are the biochemical markers that are currently used in diagnosis of cardiovascular disease, follow-up observation of treatment, non-blood tests such as ECG and angiography are more usefully used. Among many biochemical markers, including aforesaid biochemical markers, that are under study for diagnosis of cardiovascular diseases that are not discussed in this study, there was a recommendation of use of CRP under physicians' decision in the USA (Hoffmeister et al. 2001 ; Kim et al. 2002 ; Park, Kang 2001 ; Frederick 2003).

In Korea, more appropriate studies are required for use of CRP in diagnosis of CVD and CHD. Examples of current biochemical markers used in screening of cardiovascular diseases are cholesterol and TG. Examples of biochemical mar-

kers that are newly used in screening of cardiovascular disease are CRP and homocysteine. Examples used in follow-up observation of treatment of cardiovascular disease are CRP and hs-CRP. Therefore, CRP may be used as a prognostic marker in predicting coronary events with patients with stable or unstable angina pectoris and in treatment of CHD.

Summary and Conclusion

The development of biochemical markers is required for an early diagnosis and treatment of cardiovascular diseases that is related with dietary habits of Korean people enjoying mixtures of traditional dietary style and westernized lifestyle. Therefore, the most efficient cost-saving biochemical markers was established in this study, through analysis of biochemical markers related with dietary habits which is susceptibly being changed in association to cardiovascular diseases from the pre-disease phase. It is thought that this biochemical marker may be used as the basic data for early diagnosis and prevention of cardiovascular diseases which may be used for Korean people.

The appropriateness of biochemical markers was reviewed by professionals (pharmaceutical areas and food/nutrition areas) and CRP was identified to be possible in Korea. When susceptibility, specificity, predictability, and cost-effect were assessed based on known available data, CRP was appointed as the most possible candidate and it was assessed to be similar or superior cost-effect efficiency in comparison to current markers. Therefore, CRP may be used as a prognostic marker in predicting coronary events with patients with stable or unstable angina pectoris and in treatment of CHD. In addition, Lp(a), fibrinogen, homocysteine, UACR and SAA were appointed as candidates and hs-CRP, UACR and SAA were assessed as cost-efficient markers.

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