

**RISKS FOR PERIPHERAL ARTERIAL DISEASE IN
THE ELDERLY WITH TYPE 2 DIABETES MELLITUS :
Their Correlation with High Sensitivity C-reactive Protein and
Ankle-Brachial Index**

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ABSTRACT

The Indonesian elderly population has been projected to increase up to about four-fold in three decades (1990-2020). As a consequence of this population trend, the increased prevalence of degenerative diseases would be inevitable; this would include the prevalence of peripheral arterial disease.

This study aims to identify the correlation of diverse risk factors, either traditional or non-traditional, with the ankle-brachial index scores, and the correlation of novel non-traditional risk factor, e.i. high sensitive C-reactive protein with the prevalence of perioheral arterial disease in the elderly, age 60-80 years old, with type 2 diabetes mellitus.

Among the 146 elderly patients with type 2 diabetes mellitus, and based on measurement of the ABI score, approximately 30.9% of them had PAD. Some traditional and non-traditional risk factors having a significant correlation with the ankle-brachial index score, were age ($r = -0.396$, $p < 0.001$ for right ABI; $r = -0.509$, $p < 0.001$ for left ABI), supine systolic blood pressure ($r = -0.268$, $p = 0.012$ for right ABI; $r = -0.267$, $p = 0.013$ for left ABI), 2-hour post-prandial blood glucose ($r = -0.252$, $p = 0.018$ for right ABI), and hsCRP ($r = -0.280$, $p = 0.011$ for right ABI; $r = -0.402$, $p < 0.001$ for left ABI); whereas other risk factors like obesity based on waist circumference and BMI, non-supine systolic blood pressure, fasting blood glucose, Hb_{A1C}, duration of diabetes, plasma lipids did not show statistically significant different odd ratios. After linear regression test for risk factors having significant correlations with ABI, age and hsCRP were found to influence the ABI scores. Based on a case-control study, risk factors which, to some extent, had statistically significant values as risk factors, include older age (≥ 70 years old; OR = 7.737 [CI = 2.515-23.805]; $p < 0.001$), relatively high supine diastolic blood pressure (≥ 90 mmHg; OR = 6.882 [CI = 0.789-60.060]; $p = 0.048$), and high concentration of hsCRP (> 3 mg/L; OR = 4.420 [CI = 1.287-15.181]; $p = 0.013$). Among these statistically significant risk factors, after logistic regression test analysis, only the age of the patient appeared to have significant influence on the prevalence of PAD.

In conclusion, this study demonstrates a negative correlation between hsCRP and ABI score; and high levels of hsCRP appeared to be a risk factor for PAD. The age of the patient, however, appeared to be the strongest risk factor for PAD.

Keywords: Peripheral arterial disease, risk factors, elderly patient, type 2 diabetes mellitus, high-sensitivity C-reactive protein, ankle-brachial index.

INTRODUCTION

Indonesian elderly population was projected to increase up to 414% in the period of 1990-2020, the most remarkable increment in the world¹. In the year of 2000, Indonesia was the fourth-leading biggest elderly population worldwide after China, India, and the USA.²

Approximately 12% of the elderly population above 65 years were found to have peripheral arterial disease (PAD) in one study.³ On the other hand, another study which used Ankle Brachial Index (ABI) score had found the prevalence of PAD in the elderly subjects with type 2 diabetes mellitus (DM) age above 40 years old and above 55 years old as 20% and 18-23% respectively.³

PAD is characterized by specific athero sclerosis marked by the existence of atherosclerotic occlusion in the lower extremity and accompanied by atherothrombotic disease.⁴ In USA, PAD affects about 12 million people and the Framingham Heart Study has found 20% of people with signs and symptoms of PAD suffered from diabetes.^{4,5}

The risk factors of PAD comprises age, gender, smoking status, hypertension, hyperlipidemia, DM, and inflammation. Some specific markers of atherosclerosis that is related to those risk factors can be detected in the peripheral blood and include homocystein, lipoprotein a (Lp[a]), fibrinogen, high sensitivity C-reactive protein (hs-CRP), soluble Fas, VCAM-1, ADMA and plasminogen activator inhibitor (PAI).^{6,7,8,9}

High sensitivity C-reactive protein is one of the acute phase protein produced by the liver, mainly the hepatocytes and in the human is so-called calcium-dependent ligand binding protein because of the high affinity of this protein to bind with the phosphocholine (PC) residual. hs-CRP would also aggregate the structure which possess any relation with the ligand. The primary effect resulting from the binding of hs-CRP with its ligand is the recognition by C1q⁷ and subsequent activation of classical complement pathway, C3,

adhesion molecule, and finally membrane attack complex. Binding of hsCRP with its ligand is also part of the host defence mechanism (against infection) and reacts to autologous antigen (whether physiologically or pathophysiologically) as the secondary effects. hs-CRP is also able to prevent autoimmunity.^{10,11,12} The premise of this study is that high concentration of hs-CRP would be useful to predict the risk of peripheral arterial disease in the elderly with type 2 diabetes mellitus.

MATERIALS AND METHODS

The subjects used in this study were recruited from patients attending the Geriatric Polyclinic Sanglah General Hospital of Denpasar in 2008.

Design of the research is case control study to identify whether elevation of high sensitive C-reactive protein is a risk factor for the prevalence of PAD in the elderly with type 2 DM.

Every geriatric patients undergoing treatment program at the Geriatric Polyclinic Sanglah General Hospital Denpasar are included as the population of this study.

The subjects of this study were elderly patients aged 60 to 80 years old with type 2 DM and undergoing treatment at Geriatric Polyclinic Sanglah General Hospital Denpasar. Calculated minimal subjects required for statistical analysis were 58 people, however every subject who met the age-criteria were also recruited in the study.

Research Hypothesis

- There is a correlation between ABI score and hs-CRP variable in the elderly population suffering from type 2 diabetes mellitus.
- In the elderly with type 2 diabetes mellitus, hs-CRP concentration is higher in patients with PAD as compared to those without PAD.

Place and Time of Research

- Geriatric Polyclinic Sanglah General Hospital Denpasar, 2008.

Population

- Targeted population : every type 2 diabetic elderly patients (age ≥ 60 years old) visiting the Polyclinic at Sanglah General Hospital of Denpasar.
- Reachable population : elderly patients with type 2 DM age 60 to 80 years old whose able to be evaluated by using ABI and visiting the Geriatric Polyclinic.
- Targeted Population : elderly patients with type 2 DM age 60 to 80 years old and suffering from PAD.
Sampling (*intended sampling*) : *consecutive sampling* of population with inclusion and exclusion criteria.

Case and Control

- Case : elderly patients with type 2 DM (age 60 to 80 years old) suffering from PAD as evaluated using ABI score (less than 0.9).
- Control : elderly patients with type 2 DM (age 60 to 80 years old) without PAD.

Research Samples

The number of Case Control Samples

By using the related formula as listed below :

- By Odds Ratio which is estimated at about 3.8 with PAD and control group ratio > 1 , also control plasma hs-CRP proportion of 0.4, then the minimal sample size required for each groups is 39, and subsequently rounded to 40.

Inclusion and Exclusion Criteria

Inclusion Criteria :

- Elderly patients with type 2 DM age ≥ 60 years old whose undergoing treatment program in Geriatric Clinic Sanglah Hospital
- Willing and agree to participate in the study as subjects by signing the informed consent.

Exclusion Criteria :

- Elderly patients (age ≥ 60 years old) with acute cardiovascular attack / acute coronary syndrome, stroke, acute infection / inflammation, acute liver function disturbance (SGOT and SGPT ≥ 2 -fold higher of normal value

and chronic liver disease / hepatitis cirrhosis), neoplastic disease, acute kidney failure, and chronic kidney disease with creatinine serum > 3 mg/dL or if CCT score < 60 .

- Technically unable to undergo ABI evaluation, for instance patients with amputated extremities.

Variable Identification and Classification.

- Independent variables: risk factor, i.e. hs-CRP.
- Dependent variables : effect, i.e. PAD as measured using ABI.
- Control variables : consists of DM, hypertension, dyslipidemia, and smoking habit.
- Confounding variables : consists of genetic, coagulation factor, etc, which due to many reasons are not evaluated in this research. Samples were obtained based on consecutive sampling method from the targeted population which passed the inclusion and exclusion criteria.

RESULTS

Table 1. Abnormal Prevalence of Various PAD

Risk Factors in the Elderly with type 2 DM

| Variables | Prevalence (%) |
|--------------------------------------------------------------------------------------|----------------|
| Age (≥ 70 years old) | 31,4 |
| Central obesity (waist circumference ≥ 90 cm for male; ≥ 80 cm for female) | 69,6 |
| Obese according to BMI (≥ 25 kg/m ²) | 42,0 |
| Lying hypertension ($\geq 140/90$ mmHg) | 54,3 |
| Sitting hypertension ($\geq 140/90$ mmHg) | 58,6 |
| Standing hypertension ($\geq 140/90$ mmHg) | 52,9 |
| High level of total cholesterol (≥ 240 mg/dl) | 17,1 |
| High level of LDL cholesterol (≥ 130 mg/dl) | 42,9 |
| Low level of HDL cholesterol (< 40 mg/dl for male; < 50 mg/dl for female) | 37,1 |
| High level of triglycerides (≥ 200 mg/dl) | 21,4 |
| Fasting blood glucose with poor control (≥ 126 mg/dl) | 35,7 |
| 2-hour post prandial blood glucose with poor control (≥ 180 mg/dl) | 52,9 |
| A1C with poor control ($\geq 8\%$) | 32,9 |

Data from Perkeni 2006?

Table 2. Age Correlation and a Number of PAD Risk Factors

| Variables | Age | |
|-------------------------------|-------|-------|
| | r | P |
| Lying systolic blood pressure | 0,140 | 0,125 |
| 2-hour pp blood glucose | 0,059 | 0,313 |
| hs-CRP | 0,187 | 0,066 |

*Significant. Statistical analysis by using *Pearson correlation* dan *Spearman correlation*.

Table 3. Correlation between Various Extent of PAD Risk Factors and ABI

| Variables | Right ABI | | Left ABI | |
|-----------------------------------|---------------|-------------------|---------------|-------------------|
| | r | p | r | p |
| Age | -0,396 | <0,001* | -0,509 | <0,001* |
| Waist circumference | 0,106 | 0,388 | 0,054 | 0,660 |
| Body Mass Index | 0,169 | 0,082 | 0,158 | 0,097 |
| Lying systolic blood pressure | -0,268 | 0,012* | -0,267 | 0,013* |
| Sitting systolic blood pressure | -0,131 | 0,139 | -0,103 | 0,199 |
| Standing systolic blood pressure | -0,179 | 0,069 | -0,193 | 0,054 |
| Lying diastolic blood pressure | -0,064 | 0,299 | -0,081 | 0,252 |
| Sitting diastolic blood pressure | 0,144 | 0,177 | 0,096 | 0,215 |
| Standing diastolic blood pressure | 0,097 | 0,213 | 0,120 | 0,161 |
| Total cholesterol | -0,104 | 0,195 | -0,012 | 0,460 |
| LDL cholesterol | -0,164 | 0,087 | 0,039 | 0,376 |
| HDL cholesterol | -0,130 | 0,141 | 0,052 | 0,334 |
| Triglycerides | 0,050 | 0,340 | 0,094 | 0,218 |
| Fasting blood glucose | 0,100 | 0,204 | 0,180 | 0,068 |
| 2-hour pp blood glucose | -0,252 | 0,018* | -0,128 | 0,146 |
| A1C | 0,007 | 0,477 | 0,010 | 0,469 |
| Duration of diabetes | -0,114 | 0,179 | -0,173 | 0,081 |
| hs-CRP | -0,280 | 0,011* | -0,402 | <0,001* |

*Statistically significant. Statistical analysis by using *Pearson correlation* and *Spearman correlation*.

Case Control Study

Table 4. Odds Ratio of Various Risk Factors to the Contribution of PAD Event

| Variables | Odds ratio | 95% CI Lower-upper | P |
|-----------------------------------------------------|------------|--------------------|--------|
| Age (60-69 vs. 70-80 years old) | 7,737 | 2,515-23,805 | <0,001 |
| Waist circumference (normal vs. obese) | 0,890 | 0,346-2,293 | 0,809 |
| Body Mass Index (non-obese vs. obese) | 1,000 | 0,394-2,540 | 1,000 |
| Lying systolic blood pressure (normal vs. high) | 1,842 | 0,755-4,493 | 0,178 |
| Sitting systolic blood pressure (normal vs. high) | 0,902 | 0,270-2,198 | 0,820 |
| Standing systolic blood pressure (normal vs. high) | 1,224 | 0,507-2,957 | 0,653 |
| Lying diastolic blood pressure (normal vs. high) | 6,882 | 0,789-60,060 | 0,048 |
| Sitting diastolic blood pressure (normal vs. high) | 2,616 | 0,625-10,950 | 0,176 |
| Standing diastolic blood pressure (normal vs. high) | 2,250 | 0,619-8,184 | 0,210 |
| Lying hypertension | 2,053 | 0,836-5,041 | 0,115 |
| Sitting hypertension | 1,111 | 0,452-2,733 | 0,818 |
| Standing hypertension | 1,357 | 0,559-3,292 | 0,499 |
| Total cholesterol (normal vs. high) | 1,000 | 0,316-3,189 | 1,000 |
| LDL cholesterol (normal vs. high) | 2,037 | 0,834-4,976 | 0,116 |
| HDL cholesterol (normal vs. low) | 1,000 | 0,399-2,506 | 1,000 |
| Triglycerides (normal vs. high) | 0,333 | 0,105-1,059 | 0,056 |
| Fasting blood glucose (normal vs. high) | 0,722 | 0,289-1,804 | 0,485 |
| 2-hour pp blood glucose (normal vs. high) | 1,353 | 0,560-3,26 | 0,501 |
| A1C (normal vs. high) | 0,704 | 0,272-1,823 | 0,469 |
| Duration of diabetes (<5 vs. ≥5 years) | 2,087 | 0,800-5,444 | 0,130 |
| hs-CRP (normal vs. high) | 4,420 | 1,287-15,181 | 0,013 |

- From 146 elderly patients suffering from type 2 DM, the PAD prevalence are:
 - > Prevalence = 30.8%.
 - > Age 60-69 : 70 years old above = 20.79% : 48.89% (p = 0.001).
 - > There are no differences based on gender.

Correlation of Several PAD Risk Factors with ABI

- Based on this research : there is negative correlation between ABI score and age, lying SBP, 2-hour pp blood glucose concentration, and hs-CRP.
- In this research, ABI score yields the firmest correlation with age (r = -0.509; p < 0.001), and hs-CRP (r = -0.402; p < 0.001).

Correlation of Age with Several PAD Risk Factors.

- After correlation test among various PAD risk factors and ABI (left and right): there is correlation between ABI and age, lying SBP, 2-hour pp blood glucose concentration (only with right ABI), and hs-CRP.
- Lying SBP and hs-CRP also influences ABI score in elderly with type 2 DM.

Odds ratio of Various Risk Factors and its Contribution to the Development of PAD.

- In this research, the extent of risk (odds ratio [OR]), i.e. several PAD traditional and non-traditional risk factors which contribute to the event of PAD are examined.
- 40 cases of PAD and 40 cases without PAD (control) whose fulfilled the overall patient criteria as had been examined in the beginning of the research have been analyzed.
- From the result of this research, among great extents of risk factors which have been observed and statistically significant as risk factors consist of: older age (≥ 70 years old; OR = 7.737 [CI = 2.515-23.805]; $p < 0.001$), highly lying DBP (≥ 90 mmHg; OR = 6.882 [CI = 0.789-60.060]; $p = 0.048$), and high concentration level of hs-CRP (> 3 mg/L; OR = 4.420 [CI = 1.287-15.181]; $p = 0.013$).
- Another risk factors analyzed in this research do not demonstrate statistically different ORs.

DISCUSSION

Peripheral Arterial Disease

Peripheral arterial disease is a specific illness marked by atherosclerosis with arterial occlusion on the lower extremities, whereby this is one of macroangiopathy state in diabetes mellitus.^{3,13} The most common symptom arises from PAD is called intermittent claudication, which characterized by pain and paralysis of metatarsal and lower

extremities. These symptoms become more prominent while walking or during activities (walking history) and diminish after a period of rest. In a severe form of PAD or stage 4, those symptoms will strongly expressed during rest period and even accompanied with the presence of gangrene.^{3,5,14}

Atherosclerosis as a basic pathology in the Pathogenesis of Peripheral Arterial Disease

Atherosclerosis underlies the peripheral arterial disease, stroke, coronary artery disease, and aortic aneurysm. One of the developmental processes of atherosclerosis is response to injury. During normal condition, endothelial cells which lining up the tunica intima form a permeable barrier to regulate the influx of plasma substances into arterial wall. Damage to these endothelial cells, either small or relatively large would change the permeability characteristics and ability of these cells to bind one another with the connective tissues beneath.^{8,15}

Peripheral Arterial Disease and its Correlation with hs-CRP

High sensitive C-Reactive Protein (hs-CRP) is a form of an acute phase protein. CRP belongs to pentraxin family, a plasma protein which binds to its ligand. Human CRP molecule (M_r 115.135) consists of five identical non-glycosylated polypeptide subunit (M_r 23.027), each contains 206 amino acids residues.¹⁶

hs-CRP has the highest binding affinity on the phosphocholine residues¹⁷. However, it also binds to a variety of autologous and extrinsic (nuclear ribonucleoprotein and apoptotic cell) ligand. Extrinsic ligand (phospholipid, fungi, parasites) when aggregating with macromolecule ligand is recognized by C1q. This would activate the classical complement pathway, binds to C3 (adhesion molecule on complement system) and membrane attack complex terminal, C5-C9. As soon as after hs-CRP binds to its ligands, it owns a broad secondary effects, i.e. initiation, opsonization, phagocytosis, and lysis of cell as a response to inflammation.¹⁸

hs-CRP has been extensively correlated with the severity of atherosclerosis, whereas it also able to predict the cardiovascular event on whether or not cardiovascular disease-free individuals. The increased concentration level of inflammatory mediators can depict the inflammation happened in the arterial wall (which is related to atherosclerosis) and may also directly involved in the disease process. Obviously, from the pathological perspective, atherosclerosis is correlated with cytokines and typical cells responsible for inflammation. Similar to the previous studies, hs-CRP tends to increase on patients with atherosclerosis.^{19,20}

The Effects of hs-CRP Concentration in Elderly

There has been a relatively few data or researches regarding the measurement of hs-CRP concentration in elderly as a specific population study. One of those is the study conducted by Maija Hassinen et al. (2006) in Finland among 103 elderly women age 60 to 70 years old in 1991. After undergoing follow-up procedure for 12 years, a striking result was revealed. Even a little increment of hs-CRP concentration would increase the propensity of elderly women to suffer metabolic syndrome (i.e. obesity, insulin resistance, dyslipidemia, increased of blood pressure, and endothelial dysfunction) approximately 5 to 6-fold higher in the future, when compared with those who experienced a decrease in hs-CRP concentration.^{21,22,23}

hs-CRP Concentration Measurement

High-sensitivity test procedure in response of measuring the CRP concentration is recommended for people who would like to identify quantitatively the risk of cardiovascular disease. To memorize this issue, the CRP terminology that is frequently used by doctor is hs-CRP.^{15,24}

During the usage of hs-CRP as one of the risk factors of cardiovascular disease, it is classified into three different

groups based on the classification of the American Heart Association (AHA) and the Centers for Disease Control and Prevention (CDC), i.e.: Low risk: <1.0 mg/L; Average risk: 1.0-3.0 mg/L; High risk: >3.0 mg/L (API, 2005).

hs-CRP Level and Peripheral Arterial Disease in Elderly with DM

Diabetes mellitus can directly involves in the mechanism of atherosclerosis which underlies the pathogenesis of PAD.²⁵ Hiperglycemia and insulin resistance implicates in endothelial dysfunction on type 2 DM. Another conceivable-related factors comprise the increase of oxidative stress and lipid involvement, which are still argued for its role.³

Peripheral Arterial Disease has become a major cause of morbidity for elderly living in the USA. The incidence of PAD is reaching up 26.6 per 1000 male and 13.3 per 1000 female age above 65 years old. Prevalence of PAD is about 10% in general population age above 55 years old. Data obtained from The Framingham Heart Study demonstrates the co-existence of symptomatic PAD in diabetic patients for approximately 20% of study population.³ Inflammation has been recognized not only as a marker but also for its probability of becoming the risk factor of PAD in diabetes. The increase of hs-CRP is firmly correlated with the occurrence of PAD in diabetic patients.³ However, this issue was unable to be proved in this study. The results are: there is no significant difference of hs-CRP mean concentration ($p = 0.869$) between PAD and non-PAD patients. The result of analysis demonstrates an elevated risk of getting PAD to 2-fold higher in accord with elevation of hs-CRP concentration (cut point 1 mg/L) on elderly with diabetes mellitus, yet it possessed an insignificant statistical power ($p = .205$). Despite both results were insignificant, the elevation of hs-CRP with cut point of 1 mg/L seems to have better accuracy in estimating the event of PAD on elderly with DM when compared to cut point of 3 mg/L. This is probably as a result of sample inadequacy to fulfill the statistical analysis criteria or might be due to the

relatively weak internal correlation between those variables itself.

Novelty

The study regarding the role of new PAD risk factors (i.e. non-traditional) in elderly with type 2 DM is rarely conducted, whereas there has been no similar research had been performed in Indonesia.

Based on assumption of any differences in other population, including race and geographical factors, thus the research related to the role of several non-traditional risk factors that is linked to the development of PAD is observed here. To date, there has been no study able to found any correlation between risk factors that comprehensively contribute to the event of PAD.

This research found, apart of traditional risk factors, i.e. age, blood pressure, and blood glucose concentration, it is worthy to note the role of newly discovered risk factors, hs-CRP and its role on the development of PAD in elderly patients with type 2 DM.

A sum of correlations between various risk factors in elderly with type 2 DM and its role to the development of PAD has been determined.

Study Limitations

- Methodological aspect.
- The pathogenesis of PAD is relatively complex which involves various contributions of risk factors.
- Knowledge aspect.
- The pathogenesis of PAD is relatively complex with no single risk factor which has been proven to be the direct causal of PAD development.
- In this research, the extent of risk (odds ratio [OR]), i.e. several PAD traditional and non-traditional risk factors which contribute to the event of PAD are examined.
- 40 cases of PAD and 40 cases without PAD (control) whose fulfilled the

overall patient criteria as had been examined in the beginning of the research have been analyzed.

- From the result of this research, among great extents of risk factors which have been observed and statistically significant as risk factors consist of: older age (≥ 70 years old; OR = 7.737 [CI = 2.515-23.805]; $p < 0.001$), highly lying DBP (≥ 90 mmHg; OR = 6.882 [CI = 0.789-60.060]; $p = 0.048$), and high concentration level of hs-CRP (> 3 mg/L; OR = 4.420 [CI = 1.287-15.181]; $p = 0.013$).
- Another risk factors analyzed in this research do not demonstrate statistically different ORs.

CONCLUSIONS AND RECOMENDATIONS

Conclusions

A negative correlation between hs-CRP and ABI score has been found .

A highly level of hs-CRP concentration is a risk factor (4.4-fold higher when compared with normal concentration) to the development of PAD.

Important discoveries can be concluded i.e.: age (mainly for those ≥ 70 years old) is the most essential risk factor and truly consistent to the development of PAD. On the other hand, hypertension and blood glucose concentration are both weak traditional risk factors and become the most inconsistent risk factors of PAD on this research.

Recommendations

- Further studies are required to reveal another novel risk factors which possess an important role in the pathogenesis of PAD.
- PAD is the easiest diagnosed-form of atherosclerosis event which is able to predict another more fatal cardiovascular events (e.g. coronary heart disease and stroke).
- Knowledges about essential PAD risk factors among our population (Bali in

particular and Indonesia in general) in turn, become more important when related to prevention and treatment.

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