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HIGH SENSITIVITY C-REACTIVE PROTEIN SHOWING INDEPENDENT RELATIONSHIP WITH PERIPHERAL VASCULAR ATHERSCLEROSIS AND WAIST CIRCUMFERENCE IN HEALTHY ADULTS

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ABSTRACT

This study investigated the associations among serum hs-CRP, peripheral vascular atherosclerosis, body mass index (BMI), and waist circumference (WC) in 347 healthy Thai adults. Overweight, obesity, central obesity, and peripheral vascular atherosclerosis occurs in healthy adults. Decreasing 0.1 of ABI and increasing 10 cm. of WC resulted in increasing of hs-CRP by 0.4 and 0.2 mg/L, respectively (R^2 = 0.10, P = 0.013) while BMI could not be used to predict hs-CRP and vascular indexes in this study. Healthy adults with central obesity and having vascular atherosclerosis had hs-CRP (2.31 mg/L) higher than those (0.82 mg/L) without vascular atherosclerosis (P<0.001). In conclusions, a reduction in WC could be predicted in a reduction in hs-CRP and also a reduction in vascular atherosclerosis could be predicted when hs-CRP was decreased. Controlling WC and following serum hs-CRP may help guide preventive interventions to reduce future CVD in healthy Thai adults.

KEYWORDS:Inflammatory markers, peripheral artery disease, atherosclerosis, central obesity, vascular stiffness, vascular occlusion

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INTRODUCTION

C-reactive protein (CRP) is a plasma acute phase protein commonly called high sensitivity C-reactive protein (hs-CRP) because of its increasing sensitivity of methodology used in the clinical laboratory at the very low concentration [1]. Hs-CRP concentration higher than 3 mg/L indicates high risk of cardiovascular disease (CVD) [2]. Several evidences show that hs-CRP can be used to predict CVD, including peripheral artery disease (PAD). Furthermore, decreasing hs-CRP and low density lipoprotein cholesterol (LDL) levels have more clinical usefulness in CVD treatment [3,4,5]. Vascular stiffness and occlusion are risk indicators for peripheral vascular blockage that can cause PAD [6]. PAD is the disease of blood vessels outside the heart and brain that is caused by the narrowing of vessels that carry blood to the legs, arms, stomach, or kidneys. People with PAD often have fatty build up in the arteries of the heart and brain. The severity of PAD correlated to serum hs-CRP that showed a relation to future CVD in PAV patients [7]. Ankle-Brachial Index (ABI) is the noninvasive index for evaluating occlusion at the extremities that are from the ankle to the center. ABI lower than 0.9 shows the association with occlusion in peripheral arteries [8]. Brachial-ankle pulse wave velocity (PWV) is an index for evaluating stiffness. PWV was increased in atherosclerosis [9]. There is little existing information that shows association among hs-CRP, WC, BMI, and vascular atherosclerosis in healthy Thai adults. There are several changes in high technologies, convenience, and food in rural Thailand that impact people's lifestyles including little knowledge about risk factors in CVD in rural Thai people. This study aims to assess obesity, central obesity, and peripheral vascular atherosclerosis and to determine the relationship of hs-CRP, body mass index (BMI), waist circumference (WC), peripheral vascular atherosclerosis, and other blood biochemical markers in healthy Thai adults in rural Thailand.

MATERIALS AND METHODS

(i) Study population

The subjects with the following criteria were included into the study: non-smoking, age ≥40 years, no recent illness, conditions such as tissue injury, infection, autoimmune disease, cancer, general inflammation or chronic inflammation, and taken any medication. The subjects who could not take blood specimens or didn't provide the consent form were excluded from the study. Medical histories of each subject were obtained by a questionnaire at the time of health examination at Bang Krang Primary Care Unit, Budhachinaraj Hospital, Phitsanulok, Thailand. The general information such as CVD or smoking history, age, BMI, WC, and blood pressure (BP) were collected. Subjects who pass the criteria will be defined as healthy adults. This study was approved by the Institute Review Board of the Naresuan University for Human Research Study.

(ii) Classifications of obesity, non-central obesity, and central obesity

Normal weight, overweight, and obesity defined by BMI<25.00, BMI=25.00-29.99, and BMI≥30, respectively. Non-central obesity was defined when WC is < 90 cm and < 80 cm for men and women, respectively, while central obesity was defined when WC is equal or exceeds above [10].

(iii) Biochemical determinations

Fasting blood samples were collected twice. The first 10 mL of blood was tested for plasma glucose, serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and hs-CRP. A second 5 mL obtained two weeks later was determined for only hs-CRP. These parameters were performed using an automated clinical chemistry analyzer (OLYMPUS AU 640, Olympus Corporation, Tokyo, Japan). The hs-

CRP concentrations of individual subject were averaged and used for data analysis.

(iv) The measurement of brachial-ankle pulse wave velocity (PWV) and ankle brachial index (ABI): Non-invasive vascular parameters, PWV and ABI, were measured using the VP-1000 Analyzer (Colin, Co. Ltd., Komaki, Japan). Details of the methodology were previously described [11]. Each subject was examined while resting in the supine position. Cuffs were wrapped on both brachia and ankles. Pulse volume waveforms at the brachium and ankle were recorded using a semiconductor pressure sensor. PWV and ABI were measured after at least a five minute rest. Vascular stiffness was assessed by using PWV. The VP-1000 software used age, gender, and disease of individuals to calculate PWV and provide peripheral vascular stiffness interpretation. The vascular occlusion was assessed using ABI and occlusion is indicated when ABI was lower than 0.9 [8,11]. Normal vascular was defined when PWV and ABI are normal, while abnormal vascular was defined when ABI or PWV or both are abnormal.

(v) Statistical analysis

All data was analyzed using SPSS program**.** The characteristic variables were presented as mean with standard deviations (SD), excepted hs-CRP that presented as median with 95% confidence interval (95%CI). Obesity, non-central, and central obesity were presented in percentages. The Mann-Whitney test was used to determine the statistic difference of hs-CRP between groups. Stepwise multiple regression was performed to investigate the relationship among hs-CRP, PWV, ABI and other independent variables.

RESULTS

Three hundred and forty seven healthy adults were enrolled in this study and the ages of participants ranged from 40 to 80 years. Fifty-five percent (191/347) were male. Baseline characteristics and biochemical measurements of all subjects are shown in Table 1.

Table 1
Anthropometric data of all subjects

Variable	Min	Max	Mean	SD
Age, years	41	80	53	9
BMI, kg/m*	17	30	24	3
WC, cm	60	106	83	10
BP, mmHg				
SBP	88	159	123	12
DBP	58	101	74	7
PWV, cm/s				
RPWV	1113	2193	1461	193
LPWV	1094	1984	1419	191
ABI				
RABI	0.83	1.28	1.06	0.08
LABI	0.80	1.28	1.07	0.08
GLU, mg/dL	68	126	91	10
(mmol/L)	(3.8)	(7.0)	(5.0)	(0.6)
TC, mg/dL	97	376	209	50
(mmol/L)	(2.5)	(9.7)	(5.4)	(1.3)
TG, mg/dL	40	638	134	93
(mmol/L)	(0.5)	(7.2)	(1.5)	(1.1)
HDL, mg/dL	20	97	51	13
(mmol/L)	(0.5)	(2.5)	(1.3)	(0.3)
LDL, mg/dL	61	270	137	36
(mmol/L)	(1.6)	(7.0)	(3.5)	(0.9)
hs-CRP, mg/L	0.12	6.74	1.33*	0.20-5.38**

Concentration is showing in median
****95% of confidential interval of hs-CRP***

BMI, body mass index; WC, waist circumference; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse wave velocity; RPWV, right extremity pulse wave velocity; LPWV, left extremity pulse wave velocity; ABI, ankle brachial index; RABI, right extremity ankle brachial index; LABI, left extremity ankle brachial index; GLU, glucose; TC, Total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high sensitivity C - reactive protein. There were 60% (207/347) of normal weight (BMI<25.00), 37% (130/347) of overweight (BMI≥25.00-29.99), and 3% (10/347) of obesity (BMI≥30). There were 45% (156/347) of central obesity and 55% (191/347) of non-central obesity. There was 41% (143/347) of normal vascular and 59% (204/347) of abnormal vascular The abnormal features were 33% (113/204) of slightly harder with slightly occlusion, 24% (83/204) of stiffness without occlusion, and 2% (8/204) stiffness with occlusion. Stepwise regression analysis among variables, hs-CRP, BMI, WC, SBP, DBP, PWV, ABI, GLU, TC, TG, HDL, and LDL was shown in Table 2. There were three final fitted models to predict hs-CRP, PWV, and ABI (P<0.05). Serum hs-CRP was associated with PWV, ABI, WC, and TG. Decreasing in 0.1 of ABI and increasing in 10 cm of WC resulted in increasing of hs-CRP by 0.4 and 0.2 mg/L, respectively. In addition, the stepwise regression analysis also shows that increasing in 1 mg/L of hs-CRP will increase PWV by 19 cm/s and will decrease ABI by 0.012.

Table 2
Stepwise regression analysis

Dependent variables	Regression model	R^2	Adjusted R^2	*P*-value
hs-CRP	1.52 + 0.002(PWV) – 4.000(ABI) + 0.020(WC) + 0.002(TG)	0.106	0.097	0.013
PWV	520 + 6.250(SBP) – 2.540(GLU) + 18.500(hs-CRP) +332.000(ABI)	0.230	0.221	0.008
ABI	1.02 + 0.0002(TG) -0.012(hs-CRP) + 0.00006(PWV) – 0.0002(TC)	0.126	0.116	0.004

hs-CRP, high sensitivity C-reactive protein; PWV, pulse wave velocity; ABI, ankle brachial index; WC, waist circumference; TG, triglyceride; SBP, systolic blood pressure; GLU, glucose; TC, Total cholesterol; R^2, coefficient of multi-regression; adj R^2, coefficient of multi-regression after adjusted. Medians of hs-CRP were compared among non-central and central obesity adults with and without abnormal peripheral vascular as shown in Figure 1. The hs-CRP in non-central obesity (0.62 mg/L) was not significant different (P>0.05) to central obesity (0.72 mg/L). However, there was significant difference (P<0.001) of hs-CRP between non-central obesity (0.95 mg/L) and central obesity (2.31 mg/L) with presented peripheral vascular atherosclerosis.

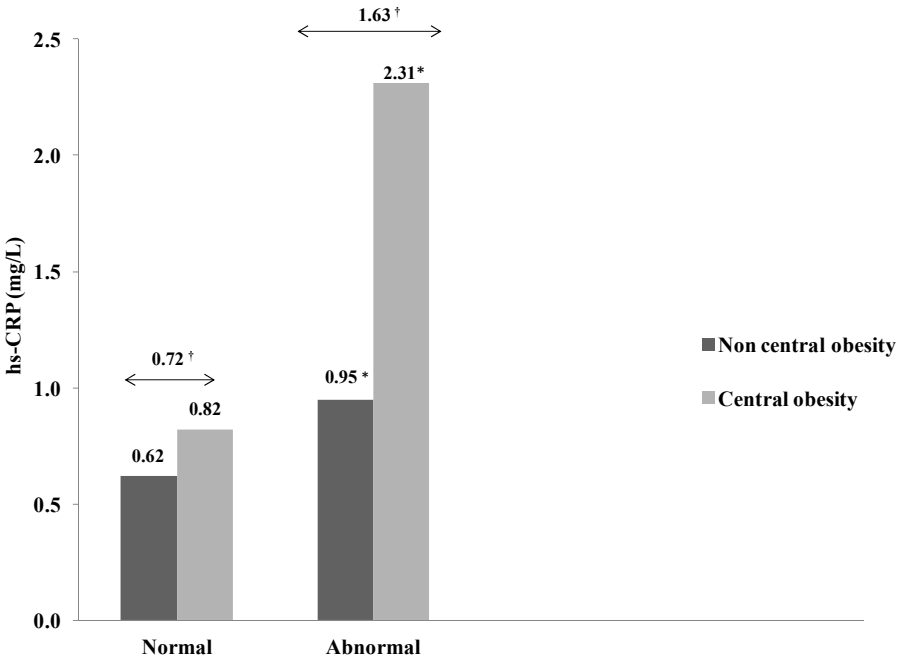

Figure 1

Median of high sensitivity C-reactive protein (hs-CRP) in normal and abnormal peripheral vascular characteristics in non-central obesity and central obesity. Peripheral vascular types were categorized by using angle brachial index (ABI) and pulse wave velocity (PWV). High sensitivity C-reactive protein concentration was determined in mg/L. Normal and abnormal were indicated normal and abnormal peripheral vascular atherosclerosis. *Significant differences between non-central and central obesity groups, P< 0.001; †Significant differences between normal and abnormal types, P< 0.001.

DISCUSSION

We measured serum hs-CRP twice, according to a previous study recommendation to ensure the reproducibility of this test, in view of biological variation [12, 13]. Any recent illness, tissue injury, infection, autoimmune diseases, cancer, general inflammation, chronic inflammation, such as arthritis, could raise the amount of CRP and falsely elevate estimations of risk. In contrast, antithrombotic medications (e.g. aspirin, cholesterol-lowering statin drugs, and ACE inhibitors) may reduce CRP. Therefore, subjects related to illnesses and medications used above were excluded from the study. The hs-CRP results were excluded if the concentration was higher than 10 mg/L [13]. The hs-CRP concentrations in healthy blood samples obtained from the automated Olympus were correlated to other 8 automated analyzers having imprecision less than 10%, except for the concentrations of hs-CRP lower than 0.15 found in only 2% of in this study [14]. hs-CRP concentration in this study (1.33 mg/L) was different than previous reports. Navapun Charuruks et. al. [15] investigated the reference value of hs-CRP in healthy volunteers from 4 center regions of Thailand (1.9 mg/L, 0.2-8.1 mg/L) which was higher than Asian women (1.12 mg/L) in the United States study [16]. There were no significant differences in the hs-CRP concentration because of region, time, and gender of age. Therefore, the determination of hs-CRP for medical purpose can be performed at any time without concerns [16] and we can use 0.2-8.1 mg/L as the reference value for hs-CRP in healthy Thai adults [15]. Peripheral vascular in healthy subjects were assessed using the non-invasive analyzer at Ban Krang Primary Care Unit as the point of care testing. To operate the instrument for ABI and PWV measurement, skilled operators are still required for consistent and accurate results. These issues have rendered ABI unpopular in primary care, due to the perceived difficulties and time taken [17]. However, an advanced technology of the VP-

1000 analyzer allows for the oscillometric to simultaneously calculate ABI by readings of blood pressure at the levels of the ankle and upper arm using specially calibrated oscillometric modules. This allows the measurement of ABI to be standardized and makes it accessible to the point of need. Most people with PAD have a higher risk of death from heart attack and stroke [18,19, 20].

PWV and ABI are widely used for assessing vascular stiffness and occlusion. Brachial- ankle PWV was measured using a volume plethysmographic apparatus form ABI. The VP-1000 Analyzer provided PWV and ABI along with interpretations using the individual information of subject such as age, gender, and disease status. The ABI below 0.9 at rest is generally considered abnormal. The validation of ABI obtained from this method has been reported previously; the inter-observer coefficient of variation (CV) was 8.4% and the intra-observer CV was 10.0% [11]. Increasing of WC was associated with increased hs-CRP concentrations. This association may be due to increasing WC as in central obesity has been associated with visceral adipose mass and a group of metabolic risk factors, such as hyperinsulinemia, dyslipidemia, and an expression of the pro-inflammatory gene tumor necrosis factor-α [21-26]. Several studies have shown that hs-CRP is correlated with BMI and visceral adiposity [21-25]. However, this study shows that hs-CRP was associated with PWV, ABI, WC and TG in healthy people and was not associated with BMI and other variables such as SBP, DBP, GLU, TC, LDL, and HDL. BMI of all subjects in this study ranged from 17-30 kg/m^2. WC is one component involved in metabolic syndrome classifications such as the NCEP, International Diabetes Federation (IDF), and World Health Organization (WHO) [27, 28]. Peripheral vascular stiffness and occlusion are indications of blocked peripheral blood vessels. Non-disease subjects having peripheral vascular stiffness and occlusion are at risk of PVD.

PVD leads to structural changes in the blood vessels resulting in inflammation and tissue damage. Previous study showed subjects with PAD often have fatty buildup in the arteries of the heart and brain. Because of this association, most people with PAD have a higher risk of death from heart attack and stroke [18,19,20]. This study shows that hs-CRP concentration in abnormal vascular with stiffness and occlusion was higher than those of normal vascular. This may due to CRP enhancing foam cell formation in arteries and activating complementary factors in atherosclerotic plaque, potentially leading to plaque rupture [7]. ABI compares the systolic ankle blood pressure with the systolic brachial blood pressure at the same site of body part for investigating how well the blood flows. ABI lower than 0.9 shows the association with occlusion in peripheral arteries. Falling down of ABI is related with severe occlusion and it has shown to be associated with CVD risk factors [29]. Previous studies found those with lower extremity arterial disease are 1.5 to 2 times more likely to experience a clinical CVD event [30]. PWV is based on simultaneous measuring the difference sites of blood pressure velocity of the pulse wave traveling a given distance between 2 sites of arterial system. Several studies have shown that the non-invasive PWV and ABI are reliable with high sensitivity and specificity to assess peripheral vascular atherosclerosis [31, 32]. However, some previous studies had shown variations and limitations of ABI [33, 34]. Age and gender were influenced to PWV [35]. Therefore, the VP-1000 analyzer uses age, gender, and disease of individual to correct PWV calculation before providing each interpretation. Limitations of this study are using non-invasive instrument to provide PWV and ABI to assess the vascular stiffness and occlusion, while the standard procedure is to assess the vascular stenosis is angiography or magnetic resonance imaging. This study enrolled healthy adults with no history of any diseases or denied current active diseases. BMI of all subjects in this study ranged from 17-30 kg/m^2. BMI over than 30 kg/m^2 indicated the severity of obesity and may associate with hs-CRP and peripheral vascular atherosclerosis.

CONCLUSION

In summary, overweight, obesity, central obesity, and peripheral vascular atherosclerosis still occurred in healthy adults that were not having currently active diseases. The hs-CRP was associated with increased WC, PWV, and triglyceride, but was associated with decreased ABI. Healthy adults with central obesity and having vascular atherosclerosis were at higher risk of CVD than those without having vascular atherosclerosis. Reduction in WC in healthy adults could predict in decrease hs-CRP and prevent of CVD. WC and serum hs-CRP may help guide preventive interventions to reduce future CVD in healthy Thai adults.

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REFERENCES

1. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high sensitivity C-reactive protein assay. Clin Chem, 45(12): 2136-2141, (1999).
2. Myers GL, Rifai N, Tracy RP, Roberts WL, Alexander RW, Biasucci LM, et al. CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: Report from the Laboratory Science Discussion Group. Circulation, 110 (25): e545-549, (2004).
3. Ridker PM. Clinical application of C-reactive protein for cardiovascular detection and prevention. Circulation, 107: 363-369, (2003).
4. Abdellaoui A, Al-Khaffaf. C-reative protein (CRP) as a marker in peripheral vascular disease. Eur J Vasc Endovasc Surg, 34 (1): 18-22, (2007).
5. Mora S, Musunuru K, Blumenthal RS. The clinical utility of high-sensitivity C-reactive protein in cardiovascular disease and the potential implication of JUPITER on current practice guidelines. Clin Chem, 55 (2): 219–228 (2009).
6. Newman AB. Peripheral arterial disease: insights from population studies of older adults. J Am Geriatr Soc, 48 (9): 1157-1162, (2000).
7. Yasojima K, Schwab C, McGeer EG, McGeer PL. Generation of C-reactive protein and complement components in atherosclerotic plaques. Am J Pathol. 2001; 158: 1039–1051.
8. Colin Medical Technology Corporation, Komaki, Japan. Non-invasive Vascular Screening Device VP-1000/2000 TBI Package manuscript.
9. Wada T, Kodaira K, Fujishiro K. Correlation of ultrasound-measured common carotid artery stiffness with pathological findings. Arterioscler Thromb Vasc Biol. (1994);(14):479-82.
10. Florez H, Castillo-Florez S, Mendez A, Casanova-Romero P, Larreal-Urdaneta C, Lee D, et al. C-reactive protein is elevated in obese patients with the metabolic syndrome. Diabetes research and clinical practice 2006; 71: 92-100.
11. Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res, 25 (3): 359-364, (2002).

12. Ledue TB, Rifai N. Preanalytic and analytic sources of variations in C-reactive protein measurement: implications for cardiovascular disease risk assessment. Clin Chem, 49 (8): 1258-1271, (2003).
13. Pearson TA, Mensah GA, Alexander WR, Anderson JL, Cannon RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation, 107 (3): 499-511, (2003).
14. Robert WL, Moulton L, Law TC, Farrow G, Cooper-Anderson M, Savory J, et al. Evaluation of Nine Automated High-Sensitivity C-Reactive Protein Methods: Implications for Clinical and Epidemiological Applications. Part 2. Clin Chem, 47 (3): 3418-3425, (2001).
15. Charuruks N, Laohajinda B, Rujiwanitgun S, Chaiworaporn M. Reference value for C-reactive protein and its distribution in Thai adults. Circ J, 69 (3):339-344, (2005).
16. Albert MA, Glynn RJ, Buring J, Ridker PM. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). Am J Cardiol, 93(10):1238-1242, (2004).
17. Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, et al. Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA, 286 (11): 1317-1324, (2001).
18. Criqui MH, Denenberg JO. The generalized nature of atherosclerosis: how peripheral arterial disease may predict adverse events from coronary artery disease. Vasc Med, 3 (3): 241-245, (1998).
19. Leng GC, Lee AJ, Fowkes FG, Whiteman M, Dunbar J, Housley E, et al. Incidence, natural history and cardiovascular events in symptomatic and asymptomatic peripheral arterial disease in the general population. Int J Epidemiol, 25 (6): 1172-1181, (1996).
20. Criqui MH, Langer RD, Fronek A, Feigelson HS. Coronary disease and stroke in patients with large-vessel peripheral arterial disease. Drugs, 42 (Suppl 5): 16-21, (1991).
21. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA, 282 (22): 2131-2135, (1999).
22. Hak AE, Stehouwer CD, Bots ML, Polderman KH, Schalkwijk CG, Westendorp IC, et al. Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. Arterioscler Thromb Vasc Biol, 19 (8): 1986-1991, (1999).
23. Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation, 102 (1): 42-47, (2000).
24. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR, et al. C-Reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. Circulation, 104 (2): 145-150, (2001).
25. Dehnavi RA, Roosb de A, Rabelink TJ, Pelt van J, Wensink MJ, Romijn JA, et al. Elevated CRP levels are associated with increased carotid atherosclerosis independent of visceral obesity. Atherosclerosis, 200 (2): 417-423, (2008).
26. Hotamisligil G, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α: direct role in obesity-linked insulin resistant. Science, 259 (5091): 87-91, (1993).
27. Florez H, Castillo-Florez S, Mendez A, Casanova-Romero P, Larreal-Urdaneta C, Lee D, et al. C-reactive protein is elevated in obese patients with the metabolic syndrome. Diabetes Res Clin Pract, 71 (1): 92-100, (2006).
28. Rahim MA, Azad Khan AK, Sayeed MA, Akhtar B, Nahar Q, Ali SMK, et al.

Metabolic syndrome in rural Bangladesh: Comparison of newly proposed IDF, modified ATP III and WHO criteria and their agreements. Diabetes Metab Res Rev, 1 (4): 251-257, (2007).

29. Yuanxi XU, Jue LI , Yingyi LUO The association between ankle-brachial index and cardiovascular or all-cause mortality in metabolic syndrome of elderly Chinese. Hypertension research; The Japanese Society of Hypertension, 30 (7):613-619, (2007).
30. Albert W. Tsai, Aaron R. Folsom, Wayne D. Rosamond, Daniel W. Jones. Ankle-brachial index and 7-year ischemic stroke incidence: the ARIC study. Stroke, 32:1721-1724, (2001).
31. Al-Qaisi M, Nott DM, King DH, Kaddoura S. Ankle brachial pressure index (ABPI): An update for practitioners. Vasc Health Risk Manag, 5: 833-841, (2009).
32. McDermott MM, Criqui MH, Liu K, Guralnik JM, Greenland P, Martin GJ, et al. Lower ankle/brachial index, as calculated by averaging the dorsalis pedis and posterior tibial arterial pressures, and association with leg functioning in peripheral arterial disease. JJ Vasc Surg, 32 (6): 1164-1171, (2000).
33. Allison MA, Hiatt WR, Hirsch AT, Coll JR, Criqui MH. A high ankle-brachial index is associated with increased cardiovascular disease morbidity and lower quality of life. J Am Coll Cardiol, 51 (13): 1292-1298, (2008).
34. American Diabetes Association. Peripheral Arterial Disease in People with Diabetes. Diabetes Care, 26 (12): 3333-3341, (2003).
35. Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, et al. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurements-a survey 12,517 subjects. Atherosclerosis, 166 (2): 303-309, (2003).