Preclinical evaluation of atherosclerosis

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The incidence of coronary artery disease and coronary events is increasing worldwide. Recent efforts have now focused on the preclinical evaluation of atherosclerosis using non-invasive imaging of both coronary and noncoronary arterial beds, or the identification of the nontraditional serum markers. This would identify individuals with high risk of adverse coronary events, who lack the conventional risk factors and thus help in risk stratification and therapy. These individuals will benefit most from lifestyle and risk factor modification. The role of these newer methods of risk stratification in the face of the conventional risk factors evaluation is discussed in this review.

KEY WORDS: Ankle brachial index, brachial artery reactivity testing, cardiac risk, carotid ultrasound, electron beam computed tomography, extra coronary vascular imaging, magnetic resonance coronary angiography

The American College of Cardiology (ACC) and the American Heart Association (AHA) endorse data from the Framingham Heart Study for the global risk assessment based on the traditional risk factors of age, gender, smoking, blood pressure, total and high-density lipoprotein (HDL) cholesterol. However, it has been increasingly recognized that these conventional risk factors do not fully account for the development of atherosclerotic coronary artery disease (CAD) and that around 50% of the patient risk is not explained for by these risk factors.[1] The aim of risk assessment is to determine whether simple reassurance, further lifestyle or pharmacological intervention or diagnostic testing is warranted. The goal of the additional non-invasive imaging for atherosclerosis is to categorize individuals into high or low risk for CAD so that treatment modalities can be optimized. However, it is important to recognize that these non-invasive imaging techniques to visualize the coronary tree and other markers that may predict coronary events are not substitutes for methods used to determine the traditional risk factors but have a complementary role in risk stratification in the context of the conventional risk factors.

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Imaging Techniques Used for Preclinical Evaluation of Atherosclerosis

Electron beam computed tomography (EBCT)

An association between coronary calcification and coronary death was described by John Hunter as early as 1775.[2] Fluoroscopically detected coronary calcification has now been shown to be a predictor of future coronary events.[3] EBCT is a highly sensitive tool for detection of coronary calcification. It has 95% sensitivity for the detection of coronary calcification[4] and at present is one of the strongest non-invasive tools available for measuring the total calcified plaque burden.[4] Although a highly sensitive test, it however lacks the specificity (45-50%) that is required in a screening test, which when positive may lead to further invasive tests of potential risk. Also, it is less sensitive in the detection of single vessel CAD, does not equate with site-specific stenosis[5] and lacks the ability to detect non-calcified atheromas demonstrated by intravascular ultrasound.[6] It must be recognized that the detection of prevalence of calcification (by EBCT) is much higher than the risk of event based on the Framingham risk, which implies that it may not be very useful in predicting coronary events.

Serial EBCT may become a powerful predictive tool if future lipid-lowering studies show that differences in calcium stores over time result in differences in coronary event rates. There have been three major prospective studies of EBCT in asymptomatic individuals for the prediction of coronary events, which are detailed in Table 1.

Table 1: Prospective studies of electron beam computed tomography in asymptomatic individuals

Study	Annualized event rate (%)	CAC* Prevalence (%)
Arad *et al*, 1996 (n=1,173)	1.5	11.8
Secci *et al*, 1997 (n=326)	1.5	50
Detrano *et al*, 1996 (n=1196)	1.6	68

*Carotid artery calcification

However, it is not currently recommended as a screening tool in young (<40 years old) healthy individuals without risk factors.

Magnetic resonance coronary angiography

Magnetic Resonance (MR) coronary angiography has developed rapidly over the past several years. However, there are several technical hurdles to be crossed still.

MR contrast enhanced oblique projection, breath-hold sequence MR has a sensitivity of around 94% and a specificity of around 57%. MR-detected coronary flow velocity / flow reserve and Doppler flow wire analysis have good correlation, making this technique potentially useful in assessing the functional significance of atherosclerotic plaques.[7]

MR coronary angiography continues to improve, but at this time its applicability for assessing cardiovascular risk still remains limited.

Extracoronary vascular imaging

Atherosclerosis is a diffuse process and usually affects most vascular beds. Hence non-invasive imaging of superficial arteries such as carotids, femorals and abdominal aorta by ultrasound has been used as a surrogate measure of coronary atherosclerosis. The number of extracoronary sites affected and the grade of atherosclerosis correlate with a greater number of 'standard' risk factors and thus with a greater cardiac risk.[8] Ultrasound can detect noncalcified atherosclerotic involvement in the form of intimal medial thickness, and hence ultrasound evaluation of multiple extra-coronary sites is a better predictor of cardiovascular (CV) events than EBCT.[9]

Carotid ultrasound

Of the peripheral arterial surrogates, carotid atherosclerosis as determined by ultrasound correlates most closely with CAD and cardiac risk within populations. Autopsy studies have shown a close histological relationship between coronary and carotid atherosclerotic disease,[9] and carotid intima-medial thickness (IMT) has been shown to be a good indicator of the presence and extent of coronary atherosclerosis in observational studies.[10,11] A direct relationship has been shown nearly universally in several studies that with increasing carotid IMT, progression to overt plaque and increasing complexity of plaque, there is an increasing chance of finding coexistent significant CAD. Also, factors that reduce 'standard' cardiac risks influence progression rates of carotid atherosclerosis as measured by ultrasound.[12-16] Carotid atherosclerosis and coronary atherosclerosis have been shown to be related to the same conventional risk factors. In a long-term follow-up of subjects in the cholesterol lowering atherosclerosis study, absolute carotid intima-medial thickness and progression of thickening predicted coronary events better than did lipid measurements.[17] Peripheral arterial ultrasonography thus is a sensitive tool for the detection of early atherosclerosis and may have an additional value to conventional risk factor assessment. It may also be useful in assessing the response to therapy.

Further investigation regarding ultrasound-stratified risk reduction therapy and cardiac outcomes would be useful in evaluating its role as a screening test.

The measurement of carotid IMT is rapid and completely noninvasive (nonionizing radiation). It has the advantage of focusing only upon the carotid artery and is done preferably in the far wall. It is most commonly measured from B-mode (two-dimensional) images with linear ultrasound transducers between 7.5 and 10 MHz. However, the small size of carotid IMT (usually less than 1 mm) necessitates computer-assisted measurement using electronic calipers. Discrete plaques can be reliably detected and localized by thorough scanning. A continuous relationship between carotid IMT and CV risk has been found: risk of myocardial infarction (MI) increased by 11% for each 0.1 mm increase in the common carotid IMT.[18]

An absolute definition of a cut-off for abnormally high IMT is problematic because of a strong influence of age and hypertension-related independent increase in IMT. So normograms based on age and other covariates in each population need to be developed to establish a level of age-adjusted relative risk which might need increased attention to CV risk factors and risk-reduction therapies. Carotid IMT is used as a common surrogate endpoint in several epidemiological studies and clinical trials to assess atherosclerotic burden, and these studies have

established that both an absolute value and the rate of progression of IMT on serial measurements are markers of adverse CV outcomes, but this requires a high degree of reproducibility.

Reproducibility studies indicate intrareader differences in Carotid-IMT are in the range of 0.04 mm. These can be reduced by the use of more modern imaging equipment, digital image acquisition and analysis, use of automated edge detection devices for quantization and making multiple assessments across time.

Brachial artery reactivity testing (BART)

The degree of arterial vasodilatation in the face of a flow-mediated increase in shear stress serves as a bioassay of endothelial cell capacity to produce and release Nitric Oxide (NO). It is an indirect indicator of endothelial function, and clinical studies have shown that endothelial dysfunction can become evident well in advance of the development of clinical or anatomic manifestations of atherosclerosis. An impaired Flow-Mediated Dilatation (FMD) response may thus represent one of the earliest stages of the development of vascular disease and could be the most useful test to screen for the early stages of arterial disease, especially in children and young adults at a time when risk factor and other interventions might be the most effective.

Subject preparation: Subjects should not smoke or eat for 8 to 12 h before the study and a quiet, temperature, controlled room should be used for the procedure. Several factors can affect flow-mediated vascular responses, such as smoking, fat or caffeine ingestion, drugs, temperature, sympathetic stimuli and the phase of the subject's menstrual cycle.

Equipment: A high frequency ultrasound instrument with a vascular transducer, built-in ECG capabilities, computer equipped with software to measure changes in brachial diameter and a broadband linear array transducer (7 to 12 MHz) are required for optimal resolution. A clear visualization of both the near and the far wall lumen-intima boundaries is required before the study begins. High quality ultrasound images are essential for accurate analysis of the brachial artery FMD response.

Definition of an abnormal brachial artery response: In a normal individual, cuff placement on the distal forearm produces a vasodilator response greater than 5%, whereas placement of the cuff above the antecubital fossa results in a vasodilator response of greater than 8%. Responses less than these are considered to be abnormal. Many factors influence these vasodilator responses. Age is a potent attenuator of vasodilator responses (especially above 40 years in men and above 50 in women). Vasodilatation is also strongly and inversely dependent on the baseline arterial diameter.

Reduced brachial artery flow-mediated vasodilatation is associated with a greater likelihood of CAD. Further, the degree of brachial endothelium-dependent vasodilatation has been related to the presence of conventional CV risk factors, exercise as also the dietary habits. Treatment of the CV risk factors has been shown to improve the brachial flow-mediated reactivity.

In patients with suspected CAD, BART better discriminates presence or absence of CAD, while IMT is a better predictor of the extent of the disease. The major limitation of BART is however the inherent biologic variability in its measurement.

Ankle brachial index (ABI)

A painless, simple and inexpensive measurement, ABI is not an imaging technique. It does not detect early plaque formation or minimal stenosis but detects individuals with more advanced (although often asymptomatic) vascular disease.

An abnormal ABI is defined as a value ≤0.90. It has a sensitivity of about 90% and a specificity of about 98% for moderate or greater obstructive peripheral artery disease (PAD) on angiography.

ABI assumes importance because the traditional clinical assessments for PAD (the pulse, palpation and symptom assessment) have low sensitivity, specificity and a low predictive value. ABI can detect subclinical (asymptomatic) cardiovascular disease (CVD), and up to 40% of patients with abnormal ABI test have no symptoms.

ABI shows fair repeatability, with a 95% CI of ±16% for a single measurement, which improves to ±10% when taken as the mean of four measurements.

ABI however is generally poorly suited to serial testing and thus is more commonly used in cross-sectional population screening.

The question now to be answered is:
Is there a clinical hierarchy of atherosclerosis imaging?

Several factors need to be weighed before a test is used for non-invasive preclinical assessment of atherosclerosis, which include the following:

1. Biological foundation of the test
2. Age of the population to be studied
3. Clinical availability of procedure
4. Reproducibility
5. Extent of prospective data demonstrating incremental information to the global risk assessment

BART, for example, is sensitive over a wide spectrum from risk predisposing factors to advanced disease and best separates normal from the abnormal in the young. ABI, on the other hand, is abnormal only in the settings of PAD and tends to cluster among patients with more advanced coronary atherosclerosis. Coronary CT detects calcium and is hence useful only in advanced plaques seen in older individuals.

Atherosclerosis Imaging in Special Groups

Women

The relationship between IMT and CV outcomes is stronger in women as shown by the ARIC Study. Lower calcium scores in coronary CT are associated with greater CV risk in females as compared with males. Also females have greater vasodilatory response than men.

Diabetes mellitus

ABI and carotid ultrasound confer greater CV event risks. BART gives more false negative results in early diabetes with hyperinsulinemia.

Ethnicity

Blacks have worse vasodilator responses on BART, less coronary calcium on CT but greater CV risk and are twice likely to have abnormal ABI than whites. Ethnic differences are minor on carotid ultrasound imaging.

Age

BART and MR are better modalities in the young, whereas coronary CT, ABI are better in older individuals.

Most of the published data for carotid ultrasound is in the middle aged and older population, its predictive value under 45 years of age is yet to be demonstrated.

How do we select patients for atherosclerosis imaging?

The global CV risk assessment in all adults is done by the Framingham model based on age, gender, smoking, blood pressure (BP), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C). Further, non-invasive imaging is to be considered in the intermediate risk group for Coronary Heart Disease (CHD). The goal is to identify individuals at 'high risk' and 'low risk' for CHD. Only ABI and carotid IMT have been shown to provide information beyond the traditional risk factors, others have potential but clear data for them is not available.

Atherosclerosis Imaging in Diabetes Mellitus (DM)

DM is a CHD risk equivalent and so diagnosis of subclinical CHD may not shift management strategy in diabetic subjects. The presence of subclinical atherosclerosis in diabetes by carotid ultrasound detects a significant gradient of CV risk. Subclinical atherosclerosis also increases the risk for incident CHD by 100%.

ABI in diabetes

Using population-based data in Chennai in a total of 1,262 subjects aged 20 years, the prevalence of PVD as defined by an ABI <0.9 was found to be 2.7, 2.9 and 6.3% in individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and diabetes respectively. Known diabetes subjects had a higher prevalence of PVD (7.8%), compared to newly diagnosed diabetes subjects (3.5%).[19]

Unpublished clinic-based data from GTBH, Delhi, from a total of 364 diabetes subjects (165 males and 199 females) with a mean age of 49.31 ± 11.90 years, mean duration of diabetes of 5.22 ± 5.24 years found an ABI <0.9 in 50 (13.73%) subjects. ABI was found to be less than 0.7 in 6 (1.64%) subjects.

The positive predictive value of ABI for CAD was found to be around 30%.

Atherosclerotic vascular disease in patients with type 2 DM

A study undertaken at our institute found significant atherosclerosis in 10% diabetics. Also, it found that an increased carotid IMT was associated with male gender, greater duration of diabetes, presence of microvascular complications. No correlation of carotid IMT was found with age, obesity, glycemic and lipid parameters or indices of insulin resistance.[20] Results are shown in Figure 1.

Another study of preclinical atherosclerosis[21] in 140

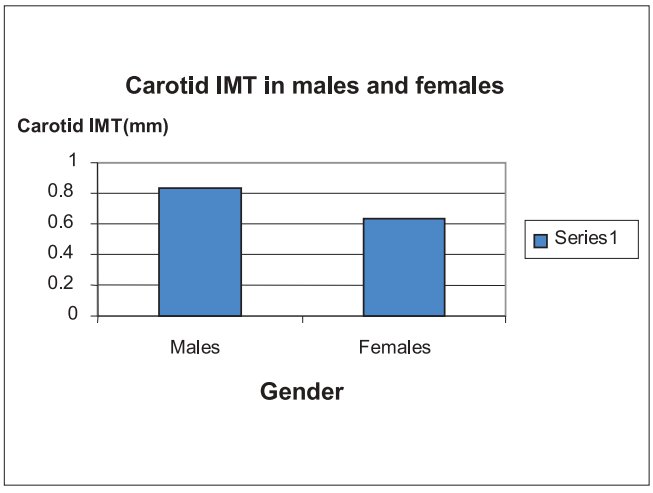

Figure 1: Carotid intima-medial thickness (IMT)

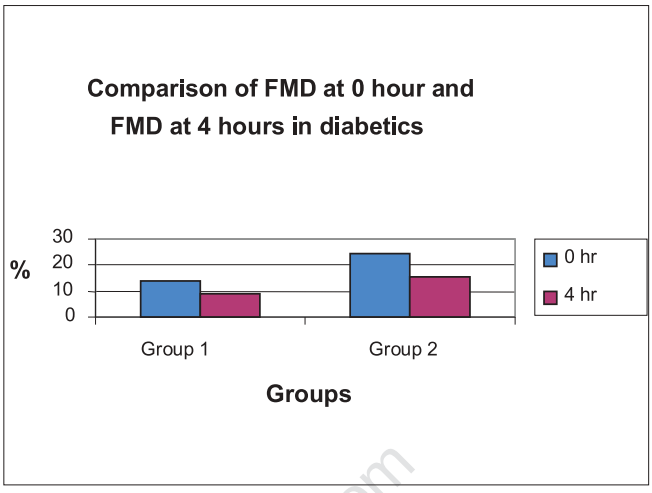

**Figure 2: Group 1: Type 2 diabetic subjects with macrovascular disease
Group 2: Type 2 diabetic subjects without macrovascular disease**

diabetic and 103 non-diabetic subjects found carotid IMT to be higher in diabetic than non-diabetic subjects. Also, diabetes and age were found to be the most important risk factors for preclinical atherosclerosis as evidenced by carotid IMT.

In a comparative study of arterial stiffness and BART *vs.* carotid IMT in 50 diabetic and 50 non-diabetic subjects, at any age higher arterial stiffness and lower FMD values were found in diabetic as compared to the non-diabetic subjects. Functional changes as evidenced by FMD correlated well with the structural changes detected by carotid IMT.

Postprandial FMD and endothelial dysfunction in patients with T2DM

In another study at GTBH,[22] it was found that FMD values were significantly lower in the postprandial state (FMD 4 hr) as compared to the fasting state in both diabetics as also healthy controls. Postprandial FMD at 4 hr was significantly lower in type 2 diabetic subjects with macrovascular disease as compared to those without macrovascular disease as also healthy controls, indicating a greater endothelial dysfunction in them after fat challenge [Figure 2].

Several novel and emerging technologies are now being developed to evaluate subclinical atherosclerosis.

Emerging technologies using nuclear imaging of vulnerable plaque

Radiolabeled monoclonal antibodies targeting molecular components of atherosclerosis, radiolabeled antibodies targeted to oxidized LDL and to components (such as apoptotic cells) of necrotic core have allowed *in vivo* nuclear imaging of atherosclerotic plaques in animal studies. Increased inflammation within symptomatic atherosclerosis can be imaged using 19 fluorodeoxyglucose as in carotid atherosclerosis. But the feasibility, accuracy and clinical utility of these technologies have not yet been demonstrated for coronary lesions or in human subjects.

Biochemical Markers for Preclinical Atherosclerosis

Infection, inflammation and hemostasis

The degree of atherosclerosis does not absolutely predict the chance of a cardiac event. Other factors are involved in plaque destabilization, thrombosis and ultimately unstable coronary syndromes or MI. Attempts to map these 'hidden factors' in atherosclerosis have now focused on infection, inflammation and hemostasis.

The role of infections, particularly chlamydia, in atherosclerosis is controversial and cannot be claimed until the completion of ongoing antibiotic intervention trials like ACES and WIZARD. Generalized screening for infectious pathogens as part of risk assessment for CAD cannot thus be advocated at present.

Whether systemic inflammation fosters atherosclerosis or whether the atherosclerotic process is, in and of itself, an inflammatory process has not been fully defined. Ruptured plaques are characterized by an inflammatory process. Data from the physicians' health study suggested that men with baseline levels of CRP in the highest quartile had a three-fold risk of a first myocardial

infarction compared with those with CRP levels in the lowest quartile. Similar data have emerged from other studies like the MONICA women's health initiative. This risk was found to be independent of other conventional risk factors, including lipids. However, altering CRP has not correlated prospectively with a change in cardiovascular morbidity or mortality. Hence routine measurement of CRP is currently not recommended by AHA or ACC.[23] It seems prudent, however, to include CRP in our standard preventive cardiac evaluation because higher CRP levels have been repeatedly and independently linked to an increased risk of the first CV event, as also its detection can lead to a change in treatment that may modify the risk for a CV event.

Other acute phase reactants like fibrinogen[24] and hemostatic factors like von willebrand factor,[25] plasminogen activator-inhibitor[26] are among the many others which have been implicated as potential markers of atherosclerotic risk. These may contribute to our understanding of the atherosclerotic process and cardiovascular risk but may not reflect modifiable risk factors. At present these markers are at best research tools only.

Regarding homocysteine, the AHA has emphasized that decisions regarding screening and treatment of patients for elevated serum homocysteine should await the results of trials currently underway in Europe and USA to evaluate the effects of lowering homocysteine on cardiovascular outcomes.[27]

Conclusion

The role of novel and emerging serum markers of increased atherosclerotic risk and the role of imaging studies are complementary, and these roles need to be precisely defined based on the current knowledge of CV risk factors. An aggressive and judicious approach should be followed in screening, diagnosing and treating atherosclerotic disease.

References

1. Braunwald E. Shattuck Lecture-Cardiovascular medicine at the turn of the millennium: Triumphs, concerns and opportunities. N Engl J Med 1997;337:1360-9.
2. Oliver MF, Samuel E, Morley P, Young GP, Kapur PL. Detection of coronary artery calcification during life. Lancet 1964;42:891-5.
3. Margolis JR, Chen J, Kong Y, Peter RH, Behar VS, Kisslo JA. The diagnostic and prognostic significance of coronary artery calcification: A report of 800 cases. Radiology 1980;137:609-16.
4. Budoff MJ, Georgiou D, Brody A, Agatston AS, Kennedy J, Wolfkiel C, *et al*. Ultrafast computed tomography as a diagnostic modality in the detection of coronary artery disease: A multicenter study. Circulation 1996;93:898-904.
5. Borman JL, Stanford W, Stenberg RG, Winniford MD, Berbaum KS, Talman CL, *et al*. Ultrafast tomographic detection of coronary artery calcification as an indicator of stenosis. Am J Card Imaging 1992;6:191-6.
6. Chou T, Redberg R, Eitetsu K, Durairaj A, Messenger JC, Sudhir K, *et al*. Screening for coronary artery atherosclerosis by detection of calcium on electron beam computed tomography: Correlation with intravascular ultrasound in asymptomatic patients with primary hyperlipidemia. Circulation 1996;94:1-360.
7. Hundley WG, Hamilton CA, Clarke GD, Hillis LD, Herrington DM, Lange RA, *et al*. Visualization of functional assessment of proximal and middle left anterior descending coronary stenoses in humans with magnetic resonance imaging. Circulation 1999;99:3248-54.
8. Simon A, Giral P, Levenson J. Extracoronary atherosclerotic plaque at multiple sites and total coronary calcification deposit in asymptomatic men. Circulation 1995;92:1414-21.
9. Mitchell JR, Schwartz CJ. Relationship between arterial diseases at different sites. A study of the aorta and coronary, carotid and iliac arteries. Br Med J 1962;1:1293-301.
10. Geroulakos G, O'Gorman DJ, Kalodiki E, Sheridan DJ, Nicolaides AN. The carotid intima-media thickness as a marker of the presence of severe symptomatic coronary artery disease. Eur Heart J 1994;15:781-5.
11. Crouse JR 3rd, Craven TE, Hagaman AP, Bond MG. Association of coronary disease with segment specific intimal-medial thickening of the extracranial carotid artery. Circulation 1995;92:1141-7.
12. Blankenhorn DH, Selzer RH, Crawford DW, Barth JD, Liu CH, Mack WJ, *et al*. Beneficial effects of colestipol-niacin therapy on the common carotid. Circulation 1993;88:20-8.
13. Crouse JR 3rd, Byington RP, Bond MG, Espeland MA, Craven TE, Sprinkle JW, *et al*. Pravastatin, lipids and atherosclerosis in the carotid arteries (PLAC-II). Am J Cardiol 1995;75:455-9.
14. Furberg CD, Adams HP Jr, Applegate WB, Byington RP, Espeland MA, Hartwell T, *et al*. Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. Asymptomatic Carotid Artery Progression Study (ACAPS) Research Group. Circulation 1994;90:1679-87.
15. Salonen R, Nyyssonen K, Porkkala E, Rummukainen J, Belder R, Park JS, *et al*. Kuopio Atherosclerosis Prevention Study (KAPS). A population-based primary preventive trial of the effect of LDL lowering on atherosclerotic progression in carotid and femoral arteries. Circulation 1995;92:1758-64.
16. Gostomyzyk JG, Heller WD, Gerhardt P, Lee PN, Keil U. B-scan ultrasound examination of the carotid arteries within a representative population (MONICA project Augsburg). Klin Wochenschr 1988;66:58-65.
17. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, *et al*. The role of carotid arterial intima-medial thickness in predicting clinical coronary events. Ann Intern Med 1998;128:262-9.
18. Salonen JT, Salonen R. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. Circulation 1993;87:56-65.
19. Premalatha G, Shanthirani S, Deepa R, Markovitz J, Mohan V. Prevalence and risk factors of peripheral vascular disease in a selected south Indian population. The Chennai Urban

Population Study. Diabetes Care 2000;23:1295-300.
20. Malhotra A, Madhu SV, Mehrotra G, *et al*. Relationship between postprandial lipid abnormalities and carotid intimal medial thicknessin patients with Type 2 DM. University of Delhi: MD Thesis; 2002.
21. Mohan V, Ravikumar R, Shanthi Rani S, Deepa R. Intimal medial thickness of the carotid artery in South Indian diabetic and non-diabetic subjects: The Chennai Urban Population Study (CUPS). Diabetologia 2000;43:494-9.
22. Kumar V, Madhu SV, Singh G, *et al*. Postprandial lipid abnormalities and endothelial dysfunction in patients with type 2 DM with and without macrovascular disease. University of Delhi: MD Thesis; 2005.
23. Grundy SM, Pasternak R, Greenland P, Smith S, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: A statement for healthcare professionals from the American Heart Association and the American College of Cardiology. J Am Coll Cardiol 1999;34:1348-59.
24. Ernst E, Koenig W. Fibrinogen and cardiovascular risk. Vasc Med 1997;2:115-25.
25. Folsom AR, Rosamond WD, Shahar E, Cooper LS, Aleksic N, Nieto FJ, *et al*. Prospective study of markers of hemostatic function with risk of ischemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation 1999;100:736-42.
26. Wu KK. Hemostatic tests in the prediction of atherothrombotic disease. Int J Clin Lab Res 1997;27:145-52.
27. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet and cardiovascular diseases: A statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation 1999;99:178-82.
28. O'Rourke RA, Brundage BH, Froelicher VF, Greenland P, Grundy SM, Hachamovitch R, *et al*. American College of Cardiology / American Heart Association Expert Consensus document on electron-beam computed tomography for the diagnosis and prognosis of coronary artery disease. Circulation 2000;102:126-40.

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