Macrovascular Component of Diabetes Atherosclerosis and Insulin (CUPS-18)

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Atherosclerosis

Atherosclerosis, a complicated multifactorial pathological process, affects the large and medium sized arteries resulting in macrovascular disease. Though the end-points of atherosclerosis are well defined, there is no clear explanation for the pathophysiology of atherosclerosis. However, the atherosclerotic process could be sequenced as functional changes and structural changes of the artery. The first to occur would be functional changes in the arteries leading to the loss of elasticity. Structural changes like fatty degeneration and foam cell formation occur later leading to intimal medial thickening, plaque formation, finally to clogging of the artery interfering with blood flow. The plaque eventually ruptures with consequent intraluminal thrombosis, which results in the end-points like coronary artery disease [CAD], cerebrovascular disease [CVD] and peripheral vascular disease [PVD]. [1] CAD and CVD are indeed the first and second causes of mortality worldwide.[2]

Macrovascular disease and diabetes

Macrovascular disease is one of the major causes of mortality and morbidity in diabetes, and several studies indicate that diabetic patients have 2-5 times higher death rates due to atherosclerotic disease than non-diabetic individuals.[3-5] The prevalence of all manifestations of coronary artery disease like myocardial infarction, angina and sudden death are higher in patients with type 2 diabetes mellitus.[6-7] In fact studies have reported CAD, to be present even at the time of diagnosis of diabetes and indeed even in prediabetic stages like impaired glucose tolerance [IGT].[8] It has been suggested that there is an eight years decrease in life expectancy due to diabetes.[9] The protective effect observed among pre-menopausal women disappears after diabetes sets in[10] and in fact diabetic women often have a higher risk for CAD than diabetic men.[11]

CVD and diabetes: Prospective studies have shown diabetes to be a major risk factor for cerebrovascular disease. Mortality due to stroke is twice as high among subjects with diabetes. A follow up study on 1,160 subjects in Minnesota, (mean follow up years: 3.4 years) concluded that diabetes is one of the predictors of future cardiovascular event.[12] Furthermore, the risk for reinfarction is shown to be higher in diabetic subjects compared to non-diabetic subjects.[13] A recent study on subjects who underwent vascular surgery reported diabetes to be a major risk for adverse outcomes like stroke and hospital death.[14]

CAD and diabetes: In the 1980's Kannel et al[3] was the first to demonstrate that diabetic men and women have a 3.5 to 4 fold higher risk of CAD mortality compared to non-diabetic women in the Framingham study. Follow up data for upto 24 years confirmed the higher mortality rates among diabetic subjects and also a cumulative effect of age and diabetes.[15] Co-existence of multiple risk factors among diabetic subjects has been shown to dramatically increase the mortality rates compared to non-diabetic subjects. The Organization to Assess Strategies for Ischemic Syndromes (OASIS) study showed that diabetic subjects without prior CAD had a similar risk for developing CAD as non-diabetic subjects with prior CAD.[4] This was confirmed again by the studies by Haffner et al[5,16] who proposed that the clock for CAD starts ticking from the stage of IGT itself.

PVD and diabetes: The prevalence of PVD has been shown to be higher among diabetic subjects compared to age and sex matched non-diabetic subjects.[17,18] Earlier studies from USA, UK, Greece, and the Netherlands have clearly shown that diabetic subjects have a higher risk for PVD compared to non-diabetic subjects.[19-21] Studies on Asian Indians have also demonstrated a similar higher prevalence of PVD among diabetic subjects.[22] However, the overall prevalence of PVD in Indians was lower than that reported in western studies.[22] Other risk factors for PVD in type 2 diabetic subjects are high triglycerides, low HDL cholesterol, hypertension and smoking.[23]

Epidemiology of diabetes and cardiovascular disease in Indians

Earlier studies in Western countries have confirmed that the prevalence of diabetes among migrant Indians is significantly higher than in the indigenous populations.[24,25] In the 1970's diabetes among urban Indians was shown to be 2.1%, [26] this rose to 8.0% in 1982[27] and is currently 12% according to the recent National Urban Diabetes Study.[28] Recent studies from different regions of the country have shown a rising prevalence of diabetes.[29,30] Projections by WHO,[31] have highlighted that India already leads the world in the prevalence of diabetes and would continue to hold the top position in the forthcoming years and numbers are expected to increase to 80.9 million by the year 2030.[32]

The Chennai Urban Population Study (CUPS)

To determine the prevalence of diabetes and macrovascular disease in South Indians, we undertook a population-based study in urban South Indians called the Chennai Urban Population Study (CUPS).[33] Briefly, CUPS is a population based study involving two residential areas representing the lower and middle income group in Chennai (formerly Madras) in South India. All individuals aged greater than 20 years living in these two colonies were requested to participate in the study. Of the total of 1399 eligible subjects (age ≥ 20 years), 1262 (90.2%) participated in the study. The study subjects underwent a glucose tolerance test (GTT)

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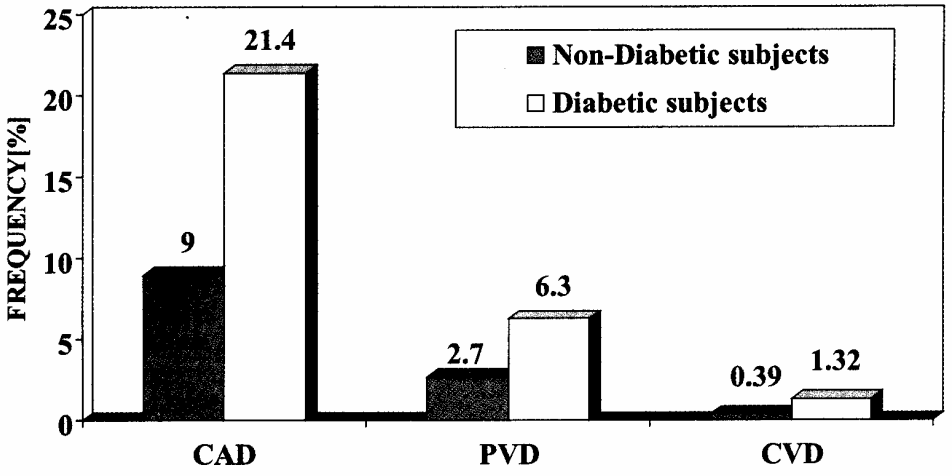

Fig. 1: *Prevalence of Macrovascular Disease - The Chennai Urban Population Study [CUPS]*[36,38]

and were categorized as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or diabetes. 12 lead ECG was also performed and CAD was diagnosed based on previous medical history of CAD and / or Minnesota coding of ECGs.[33,34]

Prevalence of diabetes: Overall, 12% of the total population had diabetes and on age standardization this reduced to 9.3%. Of the 152 diabetic subjects identified in the CUPS study, 7.2% were known diabetic subjects and 4.8% were undiagnosed diabetic subjects. The prevalence of impaired glucose tolerance was 5.9% (age standardized prevalence 5.0%). This means that 1 out of six persons in urban areas in India will have some degree of glucose intolerance. In adults aged 40 years and above, one out of four individuals will have glucose intolerance.[35]

Prevalence of CAD: The overall crude prevalence rate of CAD was 11.0%, and the age-standardized figure was 9.0%. The prevalence of CAD was higher among diabetic subjects [21.4%] compared to 14.9% in those with IGT and 9.1% in subjects with normal glucose tolerance[36] (Figure 1). Indeed the risk for CAD seemed to increase even at the stage of IGT and was similar to that noted among newly diagnosed diabetic subjects[37].

Prevalence of PVD: PVD was present in 3.2% of the study population. The age standardized prevalence in the population was 2.0%. 2.7% of the normals, 2.9% of the subjects with IGT and 6.3% of the diabetic subjects had PVD [Figure 1]. Prevalence of PVD in newly diagnosed diabetic subjects was 3.5% compared to 7.8% in known diabetic subjects.[38]

Prevalence of CVD: Self reported stroke was assessed in the CUPS population. The overall prevalence of stroke was 0.63% and in diabetic subjects it was 1.32% which was three times higher than that observed in normal subjects (0.39%) [Figure 1].

Preclinical atherosclerotic markers: Macrovascular disease is one of the clinical end points of atherosclerosis, which in its earlier stages, as discussed earlier, involves both structural and functional changes in the arteries. Structural changes can be studied using non-invasive techniques like high-resolution ultrasound by measuring Carotid Intimal Medial Thickness[IMT] while functional changes can be studied by looking at flow-mediated dilatation or arterial stiffness. These pre-clinical atherosclerotic markers have gained wide recognition as they are useful surrogate markers for CAD and can also be used in studies on prevention and intervention of CAD.[39,40]

In the CUPS study we examined the carotid intimal medial thickness (IMT) in diabetic and non-diabetic subjects. The mean IMT values among diabetic subjects were higher compared to normal subjects.[40] The range of IMT values in non-diabetic subjects was 0.5 – 1.2 mm and in diabetic subjects, 0.4 – 3.0 mm. Carotid atherosclerosis (defined as IMT >1.1 mm) was present in 20% of diabetic subjects compared to 1% of non-diabetic subjects in CUPS [Figure 2].

We also observed that the diabetic subjects had increased IMT at every age point compared to their non-diabetic counterparts.[41] Further, the newly diagnosed diabetic subjects had significantly higher IMT values compared to normal subjects, but significantly lower compared to known diabetic subjects. Further analysis of the data revealed that diabetes per se was an important risk factor for increase in IMT.[41]

We next looked at functional changes in arteries by studying endothelial dysfunction and arterial stiffness. Endothelial dysfunction was measured as flow-mediated dilatation (FMD) of the brachial artery using high-resolution B mode ultrasonography. Flow-mediated dilatation (FMD) was found to be reduced in diabetic patients (2,1 ± 2.95%) compared to age and sex matched non-diabetic subjects (6.64 ± 4.38%, $p < 0.0001$).[42]

Arterial stiffness was measured by the Augmentation index of the radial artery using the Sphygmocor machine and was found to be significantly greater in diabetic subjects (27.48 ± 7.41%) compared to age and sex matched non-diabetic subjects (19.10 ± 8.19%), $p < 0.0001$.[42]

Earlier studies on intimal medial thickness on subjects with diabetes clearly demonstrated that atherosclerosis manifests 2 to 3 decades earlier among diabetic subjects

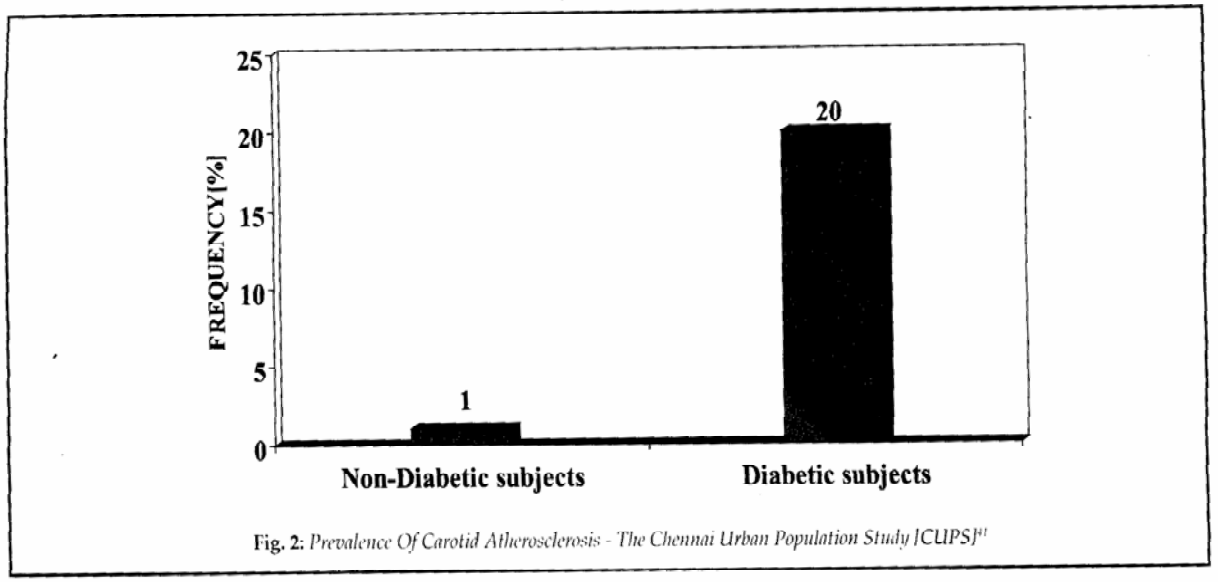

Fig. 2: *Prevalence Of Carotid Atherosclerosis - The Chennai Urban Population Study [CUPS]*[41]

compared to non-diabetic subjects.[43] The Cardiovascular Health Study also showed higher prevalence of preclinical atherosclerosis compared to normals.[44]

Insulin and atherosclerosis

Accelerated development of atherosclerosis in diabetes has been the subject of intense study in the past two decades which has enhanced our knowledge of both causative factors as well as the pathophysiology.[45] These studies have improved our understanding of the pathogenic mechanisms of atherosclerosis and the trigger mechanisms that lead to acute clinical events in diabetes. During the last two decades the role of insulin in atherosclerosis has been a subject of heated debate.

The association of insulin and atherosclerosis could be considered under two heads. The first is the contribution of increased endogenous insulin production i.e., hyperinsulinemia to atherosclerosis; second the beneficial effect of exogenous insulin in preventing cardiovascular deaths. These will be discussed in detail in the sections below.

Hyperinsulinemia, insulin resistance syndrome and atheroslcerosis: Insulin resistance and the compensatory increase in insulin secretion bring about a state of chronically increased insulin and glucose levels in the blood (hyperinsulinemia and hyperglycemia), which are predecessors for both diabetes and cardiovascular disease. The term insulin resistance syndrome includes a host of abnormalities, including hypertension, hyperinsulinemia, hypertriglyceridemia, increased levels of small dense low density lipoprotein (LDL), and low high density lipoprotein (HDL), and hypercoagulability. These abnormalities constitute the Reaven's syndrome otherwise named as Insulin Resistance Syndrome, or Pluri Metabolic Syndrome.[46]

Hyperinsulinemia and atherosclerosis: There is ample evidence to suggest a direct role of hyperinsulinemia in the development of atherosclerosis through stimulation of vascular smooth muscle cell proliferation and arterial wall lipid deposition.[47] Insulin has also been implicated as an indirect cause of atherogenesis as it promotes the development of hypertension and dyslipidemia.[48,49] The Helsinki Policeman Study, which followed up 970 non-diabetic men who were free of coronary artery disease revealed fasting insulin to be a predictor for CAD.[50]

Insulin resistance and clustering of risk factors: Although insulin resistance syndrome is a cluster of various abnormalities, a concomitant presentation of all components of the syndrome is rare. Therefore, in the view of most experts, three components are sufficient for defining the syndrome. The insulin resistance syndrome seems to explain a major part of the CAD occurrence.[51]

Insulin resistance syndrome in Indians

Hyperinsulinemia,[52] insulin resistance[53] and other components of metabolic syndrome[24,25,54] have been shown to be more prevalent among Asian Indians. The prevalence of Insulin resistance syndrome (IRS) defined using the European Group of Insulin Resistance (EGIR) criteria was found to be 11.2% among South Indians.[55] The definition for IRS was as follows: - insulin resistance calculated using the Homeostasis Model Assessment (HOMA IR), >1.93, [being the 75th percentile of the total population) in combination with at least 2 of the following conditions; hyperglycaemia, hypertension, dyslipidaemia or central body obesity.[34]

Factor analysis, a complex statistical technique has been extensively used for identifying clustering of the insulin resistance factors. Initially Meigs[56] showed that the IRS components tend to cluster together. Later, using the same technique, Lempiainen et al[57] demonstrated that this cluster predicts CAD in non- diabetic population followed up for 7 years. Studies conducted on type 2 diabetic subjects[51] and the insulin resistance syndrome cluster was also shown to predict death.[58] A similar clustering of the factors contributing to the insulin resistance syndrome has also been shown among native Indians.[59] In the CUPS study also, we observed that the metabolic abnormalities tend to cluster and that subjects with more than one metabolic abnormality had a higher prevalence of CAD compared to subjects with a single metabolic abnormality.[60] However, the role of hyperinsulinemia per se in atherogenesis is still a debatable issue and the association is seen mostly in middle aged men and not in women or in elderly men.[53]

Abnormalities of coagulation and fibrinolysis in insulin resistance: Insulin resistance is associated not only with the classic cardiovascular risk factors of hypertension and dysplipidaemia, but also with disorders of coagulation and fibrinolysis. Increased PAI-1 levels have been shown to be associated with higher insulin and proinsulin levels.[61] Patients with insulin resistance syndrome and diabetes mellitus tend to have increased PAI-1 levels. In our study on subjects with and without diabetes, PAI-1 and fibrinogen were higher among diabetic subjects compared to non-diabetic subjects.[62]

Inflammation and insulin resistance: More recently, chronic subclinical inflammation has been proposed as a part of the insulin resistance syndrome. Evidence from various studies has shown that inflammatory markers predict the development of diabetes.[63] In both insulin resistance and atherosclerosis, the acute-phase response is enhanced. Studies of the factors that regulate the acute-phase response have yielded consistent results implicating cytokines and growth factors in the pathophysiology of insulin resistance and atherosclerosis. This includes markers like C-reactive protein, interleukins and tumour necrosis factor.[64-67]

Insulin therapy and atherosclerosis

Diet and exercise are the initial therapy for Type 2 diabetic patients in whom insulin resistance is an important component of the altered glucose homeostasis. Both these forms of treatment improve the peripheral insulin sensitivity as well as the pancreatic beta cell function. If these measures do not result in the desired improvement in glycemic control, then oral agents or insulin are added. Both these forms of therapy are known to improve insulin action.

Therapeutic role of insulin – clinical benefits: Infusion of insulin has been shown to have remarkable benefit in subjects with myocardial infarction.[68-69] The Diabetes Mellitus Insulin Glucose Infusion in Acute Myocardial Infarction[DIGAMI] Study compared long term all cause mortality in 306 diabetic patients who received intensive treatment to 314 on conventional therapy.[69] The mean follow up period was 3.4 years. The study revealed a reduction in mortality by 30% in the group which received glucose insulin infusion initially, followed by four times daily insulin for three months. Recent studies have demonstrated that continuous insulin infusion reduced the mortality significantly compared to subcutaneous insulin.[70]

Therapeutic role of insulin – mechanism: Apart from controlling hyperglycaemia insulin also intervenes with many other metabolic and inflammatory pathways to prevent atherosclerotic endpoints. Plasma free fatty acids, which are increased due to enhanced catabolism, would induce inflammation and also worsen the clinical outcomes.[71] Normalizing free fatty acids by exogenous insulin could yield significant benefits.[68] Suppression of PAI-1 production by insulin is of benefit as this would increase clot dissolution.[68,72] There are many other potential benefits of insulin, mainly inhibition of pro-inflammatory early growth response gene-1(Egr-1) and tissue factor indicating its anti-inflammatory properties.[68,73] The basic mechanism by which insulin acts as an anti-inflammatory factor is by enhancing nitric oxide production.[74] The vasodilatatory effect of insulin could also be one of the reasons for its favourable effects.[75]

Conclusions

It is clear from the literature that macrovascular disease is a common complication of diabetes, the link being insulin resistance and hyperinsulinemia. On the contrary exogenous insulin therapy in diabetic subjects has shown to be beneficial. Furthermore, continuous insulin infusion is more advantageous than subcutaneous insulin. Appropriate use of insulin could help in achieving good diabetic control. Good control of diabetes, hypertension, hyperlipidaemia and obesity coupled with lifestyle changes can help to prevent atherosclerosis in diabetic patients.

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References

1. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-809.
2. Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* 1997;349:1269-1276.
3. Kannel WB. Metabolic risk factors for coronary heart disease in women: perspective from the Framingham Study. *Am Heart J* 1987;114:413-419.
4. Malmberg K, Yusuf S, Gerstein HC, Brown J, Zhao F, Hunt D, Piegas L, Calvin J, Keltai M, Budaj A. Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry. *Circulation* 2000;102:1014-1019.
5. Haffner SM. Coronary heart disease in patients with diabetes. *N Engl J Med* 2000;342:1040-1042.
6. Otel I, Ledru F, Danchin N Ischemic heart disease in type 2 diabetes : *Metabolism* 2003;52(8 Suppl 1):6-12
7. Laakso M. Cardiovascular disease in type 2 diabetes: challenge for treatment and prevention. *J Intern Med* 2001;249:225-235.
8. Taubert G, Winkelmann BR, Schleiffer T, Marz W, Winkler R, Gok R, Klein B, Schneider S, Boehm BO. Prevalence, predictors, and consequences of unrecognized diabetes mellitus in 3266 patients scheduled for coronary angiography. *Am Heart J* 2003;256:195-197.
9. Roper NA, Bilous RW, Kelly WF, Unwin NC, Connolly VM. Excess mortality in a population with diabetes and the impact of material deprivation: longitudinal, population based study. *Br Med J* 2001;322:1389-1393.
10. Marks JB, Raskin P. Cardiovascular risk in diabetes: a brief review. *J Diabetes Complications* 2000;14:108-115.
11. Sowers JR. Diabetes mellitus and cardiovascular disease in women. *Arch Intern Med* 1998;158:617-621.
12. Tsang TS, Barnes ME, Gersh BJ, Takemoto Y, Rosales AG, Bailey KR, Seward JB. Prediction of risk for first age-related cardiovascular events in an elderly population: the incremental value of echocardiography. J *Am Coll Cardiol* 2003;42:1199-1205.
13. Hillen T, Coshall C, Tilling K, Rudd AG, McGovern R, Wolfe CD; South London Stroke Register. Cause of stroke recurrence is multifactorial: patterns, risk factors, and outcomes of stroke recurrence in the South London Stroke Register. *Stroke* 2003;34:1457-1463.

14. Hagl C, Galla JD, Spielvogel D, Bodian C, Lansman SL, Squitieri R, Ergin MA, Griepp RB. Diabetes and evidence of atherosclerosis are major risk factors for adverse outcome after elective thoracic aortic surgery. *J Thorac Cardiovasc Surg* 2003;126:1005-1012.
15. Krolewski AS, Warram JH, Valsania P, Martin BC, Laffel LM, Christlieb AR. Evolving natural history of coronary artery disease in diabetes mellitus. *Am J Med* 1991;90:56S-61S.
16. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA* 1990;263:2893-2898.
17. Beach KW, Brunzell JD, Strandness DE: Prevalence of severe arteriosclerosis obliterans in patients with diabetes mellitus. *Arteriosclerosis* 1982;2:275-280.
18. Cimminiello C. PAD. Epidemiology and pathophysiology. *Thromb Res* 2002;106:V295-301.
19. Katsilambros NL, Tsapogas PC, Arvanitis MP, Tritos NA, Alexiou ZP, Rigas KL: Risk factors for lower extremity arterial disease in non insulin dependent diabetic persons. *Diabetic Medicine* 1996;13:243-246.
20. Beach KW, Bedford GR, Bergelin RO, Martin DC, Vandenberghe Zaccardi M, et al: Progression of lower extremity arterial occlusive disease in type 2 diabetes mellitus. *Diabetes Care* 1998;11:464-472.
21. Beks PJ, Mackaay AJC, de Neeling JN, de Vries H, Bouter LM, Heine RJ: Peripheral arterial disease in relation to glycaemic level in an elderly Caucasian population: the Hoorn Study. *Diabetologia* 1995;38:86-96.
22. Weerasuriya N, Siribaddana S, Dissanayake A., Subasinghe Z, Wariyapola D, Fernando DJ: Long term complications in newly diagnosed Sri Lankan patients with type 2 diabetes mellitus. *Quart J M* 1998;91:439-443.
23. Cheng SW, Ting AC, Lau H, Wong J: Epidemiology of atherosclerotic peripheral arterial occlusive disease in Hong Kong. *World J Surg* 1999;23:202-206.
24. Zimmet P, Taylor R, Ram P. Prevalence of diabetes and impaired glucose tolerance in the biracial Melanesian and Indian population of Fiji. A rural urban comparison. *Am J Epidemiol* 1983;118:673-688.
25. McKeigue PM, Pierpoint T, Ferrie JE, Marmot MG. Relationship of glucose intolerance and hyperinsulinaemia to body fat pattern in south Asians and Europeans. Diabetologia. 1992;35:785–791.
26. Ahuja MMS. Epidemiology studies on diabetes mellitus in India. In. Ahuja MMS (ed). Epidemiology of diabetes in developing countries, Interprint, New Delhi. 1979;pp 29-38.
27. Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, Das AK, Rao PV, Yajnik CS, Prasanna Kumar KM, Nair JD. High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia* 2001;44:1094-1101.
28. Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, Das AK, Rao PV, Yajnik CS, Prasanna Kumar KM, Nair JD; Diabetes Epidemiology Study Group in India (DESI). High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia* 2001;44:1094-1101.
29. Kutty VR, Soman CR, Joseph A, Pisharody R, Vijayakumar K. Type 2 diabetes in southern Kerala: Variation in prevalence among geographic divisions within a region. *N Med J India* 2000;13:287-292.
30. Misra A, Pandey RM, Rama Devi J, Sharma R, Vikram NK and Nidhi Khanna. High prevalence pf diabetes, obesity and dyslipidaemia in urban slum population in northern India. *Int J Obes* 2001;25:1-8.
31. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025-prevalence,numerical estimates and projections. *Diabetes Care* 1998;21:1414-1431.
32. Bjork S, Kapur A, King H, Nair J, Ramachandran A.Global policy: aspects of diabetes in India. *Health Policy* 2003;66:61-72.
33. Shanthi Rani CS, Rema M, Deepa R, Premalatha G, Ravikumar R, Anjana Mohan, Sastry NG, Ramu M, Saroja R, Kayalvizhi G, Mohan V. The Chennai Urban population Study (CUPS) – Methodological Details – (CUPS Paper No.1). *Int J Diab Dev Countries* 1999;19:149-157.
34. Deepa R, Shanthirani CS, Premalatha G, Sastry NG, Mohan V. Prevalence of insulin resistance syndrome in a selected south Indian population--the Chennai Urban population study-7 [CUPS-7]. *Indian J Med Res* 2002;115:118-127.
35. Mohan V, Shanthirani CS, Deepa R. Glucose intolerance (Diabetes and IGT) in a selected south Indian population with special reference to family history, obesity and lifestyle factors- The Chennai Urban Population Study (CUPS 14). *J Assoc Physicians India* 2003;51:771-777.
36. Mohan V, Deepa R, Shanthirani S, Premalatha G. Prevalence of coronary artery disease and its relationship to lipids in a selected population in South India – The Chennai Urban population Study (CUPS No. 5). *J Am Coll Cardiol* 2001;38:682-687.
37. Deepa R, Arvind K, Mohan V. Diabetes and risk factors for coronary artery disease. *Current Science* 2002;83:1497-1505.
38. 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 (CUPS). *Diabetes Care* 2000;23:1295-1300.
39. Yamasaki Y, Kawamori R, Matsushima H, et al. Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high resolution B-mode imaging. *Diabetes* 1994;43:634-639.
40. Celermajer D, Sorenson K, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects related to coronary risk risk factors and their interaction. *J Am Coll Cardiol* 1994;24:1468-1474.
41. Mohan V, Ravikumar R, ShanthiRani 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;43:494-499.
42. Ravikumar R, Deepa R, Shanthi Rani CS, Mohan V. Comparison of Carotid Intima-Media thickness, Arterial Stiffness and Brachial Artery Flow Medicated Dilatation in Diabetic and Non-Diabetic Subjects (The Chennai Urban Population Study [CUPS NO:9]. *Am J Cardiol* 2002;90:702-707.
43. Kawamori R, Yamasaki Y, Matsushima H, et al. Prevalence of Carotid atherosclerosis in diabetic patients. Ultrasound high resolution B-mode imaging on carotid arteries. *Diabetes Care* 1992;15:1290-1294.
44. O'Leary DH, Polak JJF, Kronmal RA, et al. Carotid artery intimal and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Eng J Med* 1999; 340:14-22.
45. Shwartz SM, Bornfeldt KE. How does diabetes accelerate atherosclerotic plaque rupture and arterial occlusion? *Front Biosci* 2003;8:1371-1383.
46. Reaven GM. A syndrome of resistance to insulin stimulated uptake (Syndrome X). Definitions and implications. *Cardiovasc Risk Factors* 1993;3:2-11.
47. Garber AJ. Implications of cardiovascular risk in patients with type 2 diabetes who have abnormal lipid profiles: is lower enough? *Diabetes Obes Metab* 2000;2:263 - 270.

48. Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Jarvinen H. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European Group for the Study of Insulin Resistance (EGIR). *Hypertension* 1997;30:1144-1149.

49. Garber AJ. Vascular disease and lipids in diabetes. *Med Clin North Am* 1998;82:931-948.

50. Pyorala M, Miettinen H, Laakso M, Pyorala K. Plasma insulin and all-cause, cardiovascular, and noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study. *Diabetes Care* 2000;23:1097-1102.

51. Kuusisto J, Lempiainen P, Mykkanen L, Laakso M. Insulin resistance syndrome predicts coronary heart disease events in elderly type 2 diabetic men. *Diabetes Care* 2001;24:1629-1633.

52. Mohan V, Sharp PS, Cloke HR, Burrin JM, Schemer B, Kohner EM. Serum immunoreactive insulin responses to a glucose load in Asian Indian and European Type 2 (non insulin dependent) diabetic patients and control subjects. *Diabetologia* 1986;29:235-237.

53. Sharp PS, Mohan V, Levy JC, Mather HM, Kohner EM. Insulin resistance in patients of Asian Indian and European origin with non-insulin dependent diabetes. Horm Metab Res. 1987;19:84-85.

54. Balarajan R. Ethinic differences in mortality from ischemic heart disease and cerebrovascular disease in England and Wales. *Br Med J* 1991;302:560-564.

55. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med* 1999;16:442-443.

56. Meigs JB, D'Agostino RB Sr, Wilson PW, Cupples LA, Nathan DM, Singer DE. Risk variable clustering in the insulin resistance syndrome. The Framingam Offspring Study. *Diabetes* 1997;46:1594-1600.

57. Lempiainen P, Mykkanen L, Pyorala K, Laakso M, Kuusisto J. Insulin resistance syndrome predicts coronary heart disease events in elderly nondiabetic men. *Circulation* 1999;100:123-128.

58. Lehto S, Ronnemaa T, Pyorala K, Laakso M. Cardiovascular risk factors clustering with endogenous hyperinsulinaemia predict death from coronary heart disease in patients with Type II diabetes. *Diabetologia* 2000;43:148-155.

59. Ramachandran A, Snehalatha C, Latha E, Satyavani K, Vijay V. Clustering of cardiovascular risk factors in urban Asian Indians. *Diabetes Care* 1998;21:967-971.

60. Arvind K, Pradeepa R, Deepa R, Mohan V Diabetes & coronary artery disease. *Indian J Med Res* 2002;116:163-176.

61. Nordt TK, Sawa H, Fujii S, Sobel BE. Induction of plasminogen activator inhibitor type-1 (PAI-1) by Proinsulin and Insulin in Vivo. *Circulation* 1995;91:764-770.

62. Deepa R, Velmurugan K, Saravanan G, Dwarakanath V, Agarwal S, Mohan V. Relationship of tissue plasminogen activator, plasminogen activator inhibitor-1 and fibrinogen with coronary artery disease in South Indian male subjects. *J Assoc Physicians India* 2002;50:901-906.

63. Haffner SM. Pre-diabetes, insulin resistance, inflammation and CVD risk. *Diabetes Res Clin Pract* 2003;61 Suppl 1:S9-S18.

64. Festa A, Hanley AJ, Tracy RP, D'Agostino R Jr, Haffner SM Inflammation in the prediabetic state is related to increased insulin resistance rather than decreased insulin secretion. *Circulation* 2003;108:1822-1830.

65. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC) - *Potsdam Study Diabetes* 2003;52:812-817

66. Piemonti L, Calori G, Mercalli A, Lattuada G, Monti P, Garancini MP, Costantino F, Ruotolo G, Luzi L, Perseghin G Fasting plasma leptin, tumor necrosis factor-alpha receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. *Diabetes Care* 2003;26:2883-2889

67. 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* 2000;102:42-47.

68. Dandona P, Aljada A, Bandyopadhyay A The potential therapeutic role of insulin in acute myocardial infarction in patients admitted to intensive care and in those with unspecified hyperglycemia. *Diabetes Care* 2003;26:516-519.

69. Malmberg K Prospective randomised study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. *DIGAMI (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction) Study Group BMJ.* 1997;314:1512-1515.

70. Furnary AP, Gao G, Grunkemeier GL, Wu Y, Zerr KJ, Bookin SO, Floten HS, Starr Continuous insulin infusion reduces mortality in patients with diabetes undergoing coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 2003;125:1007-1021.

71. Lundman P, Tornvall P, Nilsson L, Pernow J. A triglyceride-rich fat emulsion and free fatty acids but not very low density lipoproteins impair endothelium-dependent vasorelaxation. *Atherosclerosis* 2001;159:35-41.

72. Rauch U, Osende JI, Fuster V, Badimon JJ, Fayad Z, Chesebro JH. Thrombus formation on atherosclerotic plaques: pathogenesis and clinical consequences. *Ann Intern Med.* 2001;134:224-238.

73. Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P. Insulin inhibits the pro-inflammatory transcription factor early growth response gene-1 (Egr)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. *J Clin Endocrinol Metab* 2002;87:1419-1422.

74. Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest* 1996;98:894-898.

75. Begum N. Insulin signaling in the vasculature. *Front Biosci* 2003;8:s796-804.