Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 774–779
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Angiopoietin-2 levels in glucose intolerance, hypertension, and metabolic syndrome in Asian Indians (Chennai Urban Rural Epidemiology Study–74)

Sathish Anuradha[a], Viswanathan Mohan[a,*], Kuppan Gokulakrishnan[a], Madhulika Dixit[b]

[a]*Madras Diabetes Research Foundation & Dr Mohan's Diabetes Specialities Centre, WHO Collaborating Centre for Non-Communicable Diseases Prevention and Control, Gopalapuram, Chennai 600086, India*

[b]*Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600036, India*

Received 20 May 2009; accepted 22 September 2009

Abstract

The aim of the study was to look at the association of angiopoietin-2 (Ang-2) in Asian Indian subjects with different grades of glucose intolerance and in those with hypertension and metabolic syndrome (MS). Three groups were recruited from the Chennai Urban Rural Epidemiology Study, a population-based study in southern India, as follows: group 1, normal glucose tolerance(n = 45); group 2, impaired glucose tolerance (IGT) (n = 45); and group 3, type 2 diabetes mellitus (T2DM) (n = 40). Angiopoietin-2 was estimated by enzyme-linked immunosorbent assay. Hypertension was diagnosed based on medical history, drug treatment of hypertension, and/or if the subjects had systolic blood pressure at least 130 mm Hg and/or diastolic blood pressure at least 85 mm Hg. *Metabolic syndrome* was defined using modified National Cholesterol Education Program–Adult Treatment Panel III guidelines. Subjects with T2DM had higher age-adjusted Ang-2 values (3741 ± 1429 pg/mL) compared with subjects with IGT (1907 ± 855 pg/mL) and normal glucose tolerance (1462 ± 856 pg/mL) (P for trend < .001). Regression analysis showed that there was a linear increase in mean Ang-2 values with increasing severity of glucose intolerance, even after adjusting for age, sex, and body mass index. Angiopoietin-2 levels were also elevated in subjects with hypertension (P = .004) and in subjects with MS even in the absence of fasting hyperglycemia (P = .011). There was also a linear increase in the mean values of Ang-2 with increase in number of components of MS (P for trend < .001). This study demonstrates that increased levels of Ang-2 are seen in Asian Indian subjects with IGT, T2DM, and hypertension and in subjects with MS even in the absence of fasting hyperglycemia.

1. Introduction

Angiopoietins are a family of vascular regulatory molecules that play an important role in vascular stabilization and pathologic neovascularization [1,2]. Among the various members (angiopoietins 1-4), angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are well characterized. Angiopoietin-1 by mediating endothelial cell survival exerts a vessel sealing effect and provides protection against cardiac allograft arteriosclerosis. In contrast, Ang-2 acts primarily as a functional antagonist of Ang-1/Te-2 by binding to the receptor and blocking Ang-1–mediated endothelial signaling and thus is proinflammatory [3,4]. Increased plasma levels of Ang-2 have been reported in subjects with type 2 diabetes mellitus (T2DM) regardless of presence or absence of clinically overt cardiovascular disease (CVD) [5]. The vitreous levels of Ang-2 were also reported to be significantly higher in subjects with proliferative diabetic retinopathy [6].

Asian Indians are known to have very high rates of premature coronary artery disease and diabetes [7]. Indeed, India already has the largest number of people with diabetes in the world [8,9]. The risk for CVD starts at the stage of impaired glucose tolerance (IGT) [10,11]. However, to our knowledge, there are no data on Ang-2 in Asian Indians and none on Ang-2 levels in subjects with IGT or metabolic

No conflict of interest.

* Corresponding author. Tel.: +91 44 4396 8888; fax: +91 44 2835 0935.
E-mail address: drmohans@vsnl.net (V. Mohan).
URL: http://www.drmohansdiabetes.com (V. Mohan).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.09.022

syndrome (MS). We hypothesized that Ang-2 levels would be elevated in subjects with newly diagnosed type 2 diabetes mellitus (NDD) as well as in those with IGT. In addition, we also looked at whether Ang-2 levels are elevated in subjects with hypertension and MS.

2. Research design and methods

Study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiologic study conducted on a representative population (aged ≥20 years) of Chennai (formerly Madras), the fourth largest city in India. The methodology of the study has been published elsewhere [12]. Briefly, in phase 1 of the urban component of CURES, 26 001 individuals were recruited based on a systematic random-sampling technique, which is described in our Web site www.drmohansdiabetes.com (under the link "Publications"). Fasting capillary blood glucose was determined using a One Touch Basic glucose meter (Life scan; Johnson & Johnson, Milpitas, CA) in all subjects. Subjects were classified as "known diabetic subjects" if they stated that they had diabetes and were on the treatment [13].

In phase III, every 10th subject recruited in phase I (n = 2600) were invited to the center for detailed testing, including an oral glucose tolerance test in those without self-reported diabetes; and this had a 90% response rate (2350/2600 subjects). Those who were confirmed by oral glucose tolerance test to have 2-hour plasma glucose value of 11.1 mmol/L or more (200 mg/dL) based on World Health Organization consulting group criteria [14] were labeled as "newly detected diabetic subjects (NDD)," those with 2-hour post glucose value of at least 7.8 mmol/L (140 mg/dL) and less than 11.1 mmol/L (200 mg/dL) [14] as subjects with IGT, and those with 2-hour post glucose value of less than 7.8 mmol/L (140 mg/dL) as subjects with normal glucose tolerance (NGT).

The study subjects included for this study (from phase III) were randomly selected from the NGT, IGT, and NDD subjects and comprised the following groups (Fig. 1): group 1, 45 NGT subjects; group 2, 45 subjects with IGT; and group 3, 40 subjects with NDD who had not received any type of treatment.

2.1. Anthropometric measurements

Anthropometric measurements included weight, height, and waist and were obtained using standardized techniques as detailed elsewhere [12]. Height was measured with a tape measure to the nearest centimeter. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. Waist was measured using a nonstretchable fiber measuring tape. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Blood pressure was recorded from the right arm in a sitting position to the nearest 2 mm Hg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 minutes apart, and the mean of the 2 was taken as the blood pressure.

2.2. Biochemical parameters

Fasting plasma glucose (glucose oxidase-peroxidase method), serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method), serum triglycerides (glycerol

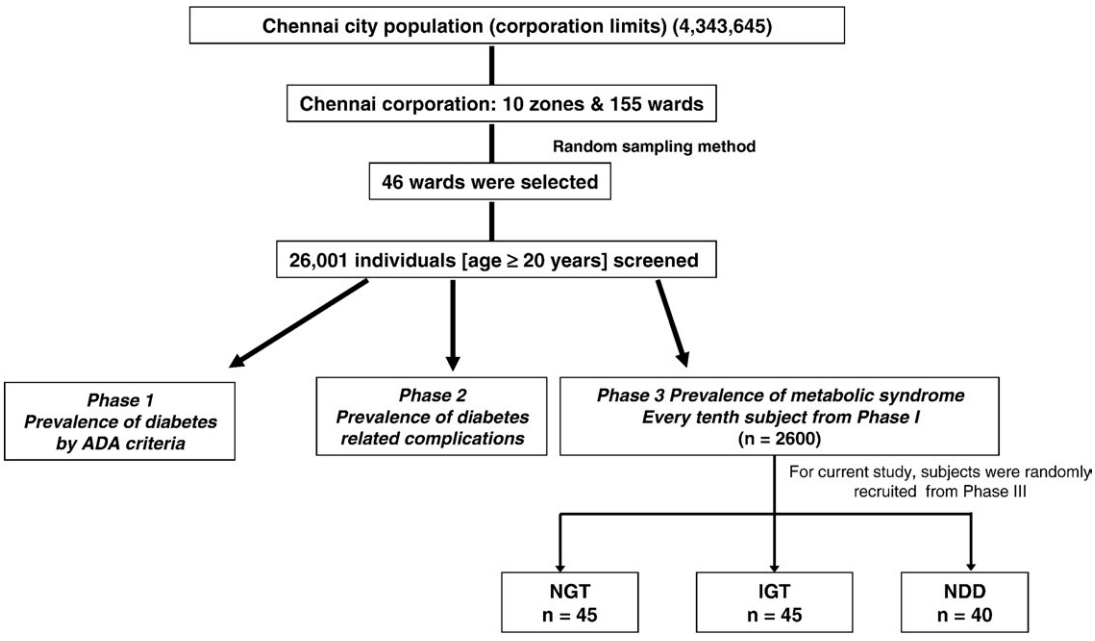

Fig. 1. Chennai Urban Rural Epidemiology Study: methodology.

phosphate oxidase-peroxidase-amidopyrine method), and high-density lipoprotein (HDL) cholesterol (direct method-polyethylene glycol-pretreated enzymes) were measured using Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany). The intra- and interassay coefficient of variation for the biochemical assays ranged between 3.1% and 7.6%. Low-density lipoprotein cholesterol was calculated using the Friedewald formula [15]. Glycated hemoglobin (HbA_{1c}) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA). The intra- and interassay coefficient of variation of HbA_{1c} was less than 10%.

2.3. Angiopoietin-2

Angiopoietin-2 was measured by enzyme-linked immunosorbent assay (R&D Systems, Abingdon, United Kingdom). In brief, monoclonal antibody specific for Ang-2 were precoated onto a microplate. Standards and samples were pipetted into the wells, and any Ang-2 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Ang-2 was added to the wells. After a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells; and color developed in proportion to the amount of Ang-2 bound in the initial step. Absorbance was read at 450 nm. The values were expressed in picograms per milliliter units. The intra- and interassay coefficients of variation were less than 5% and less than 10%, respectively.

2.4. Definitions and diagnostic criteria

2.4.1. Hypercholesterolemia

Serum cholesterol at least 200 mg/dL, hypertriglyceridemia (serum triglycerides ≥150 mg/dL), and low HDL levels (men: HDL cholesterol <40 mg/dL, women: HDL cholesterol <50 mg/dL) were diagnosed based on Adult Treatment Panel III guidelines [16].

Hypertension was diagnosed based on medical history, drug treatment of hypertension, and/or if the subjects had systolic blood pressure (SBP) at least 130 mm Hg and/or diastolic blood pressure (DBP) at least 85 mm Hg [16].

Metabolic syndrome was diagnosed based on modified Adult Treatment Panel III guidelines [16], that is, if any 3 of the following abnormalities were present: *abdominal obesity* defined as waist circumference at least 90 cm for men and at least 80 cm for women as per modified Asia Pacific World Health Organization guidelines [17], high blood pressure (SBP ≥130 mm Hg or DBP ≥85 mm Hg), elevated fasting glucose (fasting plasma glucose ≥100 mg/dL or 6.0 mmol/L), hypertriglyceridemia, or low HDL cholesterol.

2.5. Statistical analysis

Student *t* test or 1-way analysis of variance (with Tukey honestly significant difference test) was used to compare groups for continuous variables, whereas χ^2 test or Fisher exact test as appropriate was used to compare proportions. Pearson correlation analysis was carried out to determine the relation of Ang-2 with other risk variables. Multiple linear regression analysis was used to determine the association of Ang-2 with glucose intolerance. All analyses were done using Windows-based SPSS statistical package (Version 10.0, Chicago, IL), and *P* values of < .05 were taken as significant.

3. Results

The clinical and biochemical profiles of the study subjects are shown in Table 1. Subjects with diabetes and IGT were older ($P < .01$) and had significantly higher SBP and DBP ($P < .001$) compared with those with NGT. Diabetic subjects had significantly higher serum cholesterol levels compared with both NGT ($P < .001$) and IGT subjects ($P < .01$) and higher triglyceride levels compared with NGT subjects ($P < .001$).

Subjects with diabetes had higher age-adjusted Ang-2 values (3741 ± 1429 pg/mL) compared with IGT (1907 ± 855 pg/mL) and NGT subjects (1462 ± 856 pg/mL) (*P* for trend $P < .001$) (Fig. 2).

Table 2 presents the Pearson correlation analysis in the total population that reveals that Ang-2 was significantly correlated with age ($P < .001$), BMI ($P = .015$), SBP ($P < .001$) and DBP ($P < .001$), waist circumference ($P < .001$), fasting plasma glucose ($P < .001$), HbA_{1c} ($P < .001$), and serum cholesterol ($P = .023$).

Table 1
Clinical and biochemical characteristics of study subjects

Parameters	NGT (n = 45)	IGT (n = 45)	T2DM (n = 40)
Age (y)	31 ± 4	36 ± 6*	45 ± 12†,‡
Male n (%)	22 (48.9)	23 (51.1)	20 (50.0)
BMI (kg/m^2)	24 ± 4.8	25 ± 3.8	25 ± 4.3
SBP (mm Hg)	109 ± 14	121 ± 19*	136 ± 27†,§
DBP (mm Hg)	69 ± 11	77 ± 12*	83 ± 13†,‡
Waist circumference (cm)	84.3 ± 11.7	85.9 ± 11.1	88.9 ± 9.6
Fasting plasma glucose (mg/dL)	82 ± 7	90 ± 9	144 ± 59†,§
HbA_{1c} (%)	5.4 ± 0.4	5.8 ± 0.6	7.9 ± 2.0†,§
Total cholesterol (mg/dL)	172 ± 27	192 ± 39*	199 ± 38†
Serum triglycerides (mg/dL)	113 ± 60.1	153 ± 91.7	183 ± 135.8†
HDL cholesterol (mg/dL)	40 ± 7	41 ± 16	39 ± 8
LDL cholesterol (mg/dL)	109 ± 23	119 ± 31	123 ± 35

LDL indicates low-density lipoprotein,
* $P < .01$ compared with NGT.
† $P < .001$ compared with NGT.
‡ $P < .01$ compared with IGT.
§ $P < .001$ compared with IGT.

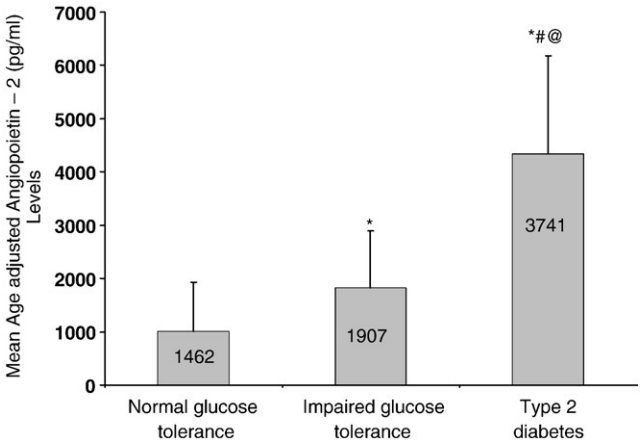

Fig. 2. Age-adjusted mean levels of Ang-2 in the study subjects. $^{@}P$ for trend < .001. *P < .001 compared with NGT. $^{\#}P$ < .001 compared with IGT

A linear regression analysis was performed, using Ang-2 as the dependent variable and glucose tolerance status as independent variable, to determine the association of different grades of glucose intolerance with Ang-2 after adjustment of age, sex, and BMI. Models were developed in such a way that only 2 groups at a time were taken up for analysis, for example, NGT and IGT or IGT and diabetic subjects. Glucose intolerance was used as the independent variable, and the group with the more severe glucose intolerance status (coded as 1) was tested against the less severe glucose intolerance status group (coded as 0 and used as reference for the analysis); for example, the NGT was used as reference for IGT, and IGT for diabetes (Table 3).

In model 1, the analysis was done in subjects with NGT (coded as 0) and IGT (coded as 1); and this was used as an independent variable. Even after adjusting for age, sex, and BMI, IGT showed a significant association with Ang-2 (unadjusted: β = 531.42, P < .001; adjusted: β = 411.4, P = .004).

In model 2, a similar analysis of subjects with IGT and diabetes was done using IGT as 0 and diabetes as 1. Even after adjusting for age, sex, and BMI, subjects with diabetes showed significant association with Ang-2 (unadjusted: β = 1885.3, P < .001; adjusted: β = 1728.5, P < .001).

Table 2
Pearson correlation analysis of Ang-2 with other risk variables

Variables	Ang-2	
	r value	P value
Age	0.5334	**<.001**
BMI	0.215	**.015**
SBP	0.384	**<.001**
DBP	0.350	**<.001**
Waist circumference	0.309	**<.001**
Fasting plasma glucose	0.454	**<.001**
HbA_{1c}	0.532	**<.001**
Serum cholesterol	0.202	**.023**
Serum triglycerides	0.164	.064
HDL cholesterol	−0.060	.498
LDL cholesterol	0.152	.086

P values in bold are <.01.

Table 3
Linear regression analysis using Ang-2 as dependent variable and varying degrees of glucose tolerance status as independent variable

Parameters	β	P value
Model 1		
Unadjusted		
(NGT = 0, IGT = 1)	531.42	**<.001**
Adjusted for age and sex	440.6	**.002**
Adjusted for age, sex, and BMI	411.4	**.004**
Model 2		
Unadjusted		
(IGT = 0, DM = 1)	1885.3	**<.001**
Adjusted for age and sex	1741.7	**<.001**
Adjusted for age, sex, and BMI	1728.5	**<.001**

P values in bold are <.001.

Subjects with hypertension had significantly higher Ang-2 values (2898 ± 1370 pg/mL) compared with subjects without hypertension (2098 ± 1228 pg/mL, P = .004). To determine whether the association of hypertension with Ang-2 was independent of hyperglycemia, we excluded all subjects with hyperglycemia and then looked at Ang-2 levels in those with and without hypertension. We found that mean Ang-2 levels were increased in subjects with hypertension (n = 3, 1691 ± 128 pg/mL) compared with subjects without hypertension (n = 42, 1445 ± 883 pg/mL) but that the differences did not reach statistical significance probably because of the small number of subjects with hypertension.

Angiopoietin-2 levels increased with increase in number of components of MS (no metabolic abnormality, 1238 ± 426; 1 metabolic abnormality, 1892 ± 1060; 2 metabolic abnormalities, 2209 ± 1207; ≥3 metabolic abnormalities, 2789 ± 1301 pg/mL; P for trend < .001) (Fig. 3).

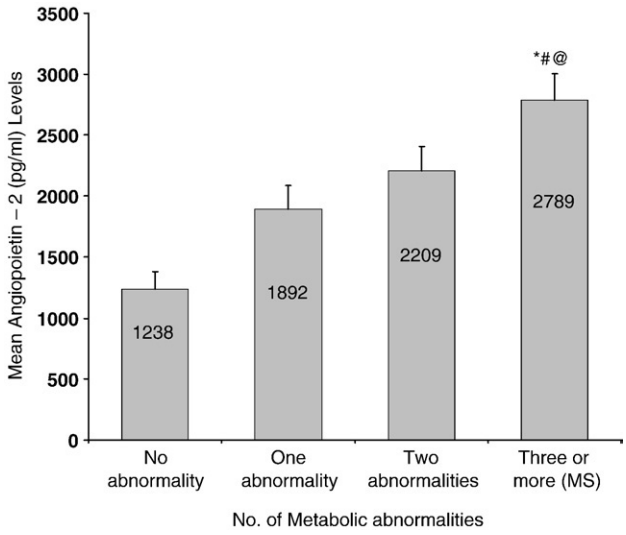

Fig. 3. Mean Ang-2 levels in relation to number of metabolic abnormalities. *P < .001 compared with no abnormality. $^{\#}P$ < .001 compared with 1 abnormality. $^{@}P$ for trend < .001.

Table 4
Mean levels of Ang-2 in those with and without metabolic abnormalities

Metabolic abnormalities		Mean Ang-2 (pg/mL)	*P* value
Elevated fasting plasma glucose	Present	3179 ± 1206	**<.001**
	Absent	1915 ± 1153	
Abdominal obesity	Present	2486 ± 1334	**.017**
	Absent	1932 ± 1334	
Hypertension	Present	2898 ± 1370	**.004**
	Absent	2098 ± 1228	
Hypertriglyceridemia	Present	2506 ± 1334	.134
	Absent	2141 ± 1263	
Low HDL cholesterol	Present	2322 ± 1348	.421
	Absent	2121 ± 1167	
MS	Present	2789 ± 1290	**<.01**
	Absent	1975 ± 1209	
MS without elevated fasting plasma glucose	Present	2743 ± 1365	**.011**
	Absent	2087 ± 1228	

P values in bold are <.001.

Mean levels of Ang-2 in subjects with and without various metabolic abnormalities are presented in Table 4. Angiopoietin-2 was higher in subjects with elevated fasting plasma glucose ($P < .001$), abdominal obesity ($P = .017$), hypertension ($P = .004$), and MS ($P < .01$). We also found that Ang-2 levels were significantly increased in subjects with MS without elevated fasting plasma glucose ($P = .011$). Although mean Ang-2 levels were higher in subjects with hypertriglyceridemia and low HDL cholesterol compared with their counterparts with normal levels of respective parameters, the differences did not reach statistical significance.

4. Discussion

The main findings in this study are that Ang-2 values increase with increasing severity of glucose intolerance, with T2DM subjects having the highest values followed by those with IGT and NGT. To our knowledge, there are no published data on the Ang-2 values in subjects with newly detected T2DM and in IGT subjects. We also report the Ang-2 values are increased in those with hypertension and MS even in the absence of fasting elevated hyperglycemia.

Subjects with diabetes are at high risk of developing micro- and macrovascular complications that are likely to mark different, but related, aspects of endothelial damage. The data presented here point to hyperglycemia as the dominant generator of the raised growth factor Ang-2 regardless of other pathologic conditions. Intriguingly, recent observations demonstrate that high glucose increases transcription of Ang-2 gene in microvascular endothelial cells [18].

Our data suggest that raised blood glucose could be one of the primary determinants of circulating Ang-2 levels. Increased blood glucose in diabetes can exert toxic effects on the endothelium through a number of mechanisms. The accelerated formation and accumulation of glycation products associated with raised blood glucose may up-regulate both Ang-2 transcription and production [19]. Therefore, our finding of selective elevation of Ang-2 and its relationship with HbA_{1c} supports these in vitro observations. In the present study, IGT subjects, who are considered to be at an increased risk for CVD [20], had higher levels of Ang-2. In the Chennai Urban Population Study, we reported that 14.7% of subjects with IGT had coronary artery disease compared with 9.0% in subjects with NGT, indicating that the higher risk for coronary artery disease is also seen in subjects with IGT [11]. However, to our knowledge, there have been no studies that have looked at levels of Ang-2 in subjects with IGT.

In the present study, among subjects with hypertension, we found raised levels of Ang-2. This is consistent with previous studies demonstrating abnormalities in markers of angiogenesis in hypertension [21-23]. Studies have shown that levels of circulating angiogenic growth factors from the angiopoietin family are specific for the endothelium and have been shown to be associated with CVD risk in subjects with hypertension [24], diabetes mellitus [25], and heart failure [26]. The increased Ang-2 levels in hypertension suggest the possibility of alterations in the whole vessel remodeling process in hypertension [27].

Glucose intolerance and other metabolic abnormalities may cluster together, resulting in increased risk for coronary artery disease [28,29]. We have observed that subjects with MS had higher levels of Ang-2 compared with subjects without MS. It was also observed that Ang-2 increased with increase in number of metabolic abnormalities, indicating a dose-response relationship between Ang-2 and metabolic abnormalities. Moreover, the values were increased even in those MS subjects without fasting hyperglycemia, showing that the association with MS is independent of hyperglycemia.

There are some limitations in this study. We have only looked at the Ang-2 levels. It would be worthwhile to study the levels of other angiogenic factors like vascular endothelial growth factor, Ang-1, and Tie-2. Secondly, being a cross-sectional study, a cause-and-effect relation cannot be determined. The strength of the study is that it is a population-based sample and the phenotypes were defined well.

In summary, this study demonstrates that increased levels of Ang-2 are seen in Asian Indian subjects with IGT, T2DM, and hypertension and in subjects with MS even in the absence of fasting hyperglycemia.

Acknowledgment

We are grateful to the Chennai Willingdon Corporate Foundation, Chennai, for the financial support provided for the study. We thank the epidemiology team members for conducting the CURES field studies. This is the 74th publication from CURES (CURES-74).

References

[1] Takagi H, Koyama S, Seike H, et al. Potential role of the angiopoietin/tie2 system in ischemia-induced retinal neovascularization. Invest Ophthalmol Vis Sci 2003;44:393-402.

[2] Jones N, Master Z, Jones J, Bouchard D, et al. Identification of Tek/Tie2 binding partners: binding to a multifunctional docking site mediates cell survival and migration. J Biol Chem 1999;274:30896-905.

[3] Hanahan D. Signaling vascular morphogenesis and maintenance. Science 1997;277:48-50.

[4] Korff T, Kimmina S, Martiny-Baron G, et al. Blood vessel maturation in a 3-dimensional spheroidal coculture model: direct contact with smooth muscle cells regulates endothelial cell quiescence and abrogates VEGF responsiveness. FASEB J 2001;15:447-57.

[5] Lim HS, Lip GY, Blann AD. Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. Atherosclerosis 2005;180:113-8.

[6] Watanabe D, Suzuma K, Suzuma I, et al. Vitreous levels of angiopoietin 2 and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Am J Ophthalmol 2005;139:476-81.

[7] Deepa R, Gokulakrishnan K, Mohan V. Coronary artery disease among Indian diabetic subjects. In: Mohan V, Rao GHR, editors. Type 2 diabetes in South Asians: epidemiology, risk factors and prevention. New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd.; 2006. p. 242-55.

[8] Sicree R, Shaw J, Zimmet P. Diabetes and impaired glucose tolerance. In: Gan D, editor. Diabetes atlas. International Diabetes Federation. 3rd ed. Belgium: International Diabetes Federation; 2006. p. 15-103.

[9] Wild S, Roglic G, Green A, Sicree R, et al. Global prevalence of diabetes, estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.

[10] Rennert NJ, Charney P. Preventing cardiovascular disease in diabetes and glucose intolerance: evidence and implications for care. Prim Care 2003;30:569-92.

[11] Mohan V, Deepa R, Rani SS, et al. 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;38:682-7.

[12] Deepa M, Pradeepa R, Rema M, et al. The Chennai Urban Rural Epidemiology Study (CURES)—study design and methodology (urban component) (CURES-I). J Assoc Physicians India 2003;51:863-70.

[13] The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus R. Report of the Expert Committee on the Diagnosis and Classification of diabetes mellitus. Diabetes Care 1997;20:1183-97.

[14] Alberti KG, Zimmet PZ. Definition diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus, provisional report of a WHO Consultation. Diabet Med 1998;15:539-53.

[15] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[16] Executive summary of the third report of the National Cholesterol Education Program (NCEP): Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). J Am Med Assoc 2001;285:2486-97.

[17] The Asia Pacific perspective: redefining obesity and its treatment. Regional Office for the Western Pacific of the World Health Organization. World Health Organization, International Association for the Study of Obesity and International Obesity Task Force. Health Communications Australia Pty Limited, Sydney. 2000; 17-21.

[18] Yao D, Taguchi T, Matsumura T, et al. High glucose increases angiopoietin-2 transcription in microvascular endothelial cells through methylglyoxal modification of mSin3A. J Biol Chem 2007;282:31038-45.

[19] Okamoto T, Yamagishi S, Inagaki Y, et al. Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. FASEB J 2002;16:1928-30.

[20] Lehto S, Ronnemaa T, Pyorala K, et al. Cardiovascular risk factors clustering with endogenous hyperinsulinemia predict death from coronary heart disease in patients with type II diabetes. Diabetologia 2000;43:148-55.

[21] Felmeden DC, Spencer CG, Chung NA, et al. Relation of thrombogenesis in systemic hypertension to angiogenesis and endothelial damage/dysfunction (a substudy of the Anglo- Scandinavian Cardiac Outcomes Trial [ASCOT]). Am J Cardiol 2003;92:400-5.

[22] Lip GY, Blann AD. Thrombogenesis, atherogenesis and angiogenesis in vascular disease: a new 'vascular triad'. Ann Med 2004;36:119-25.

[23] Nadar SK, Blann A, Beevers DG, et al. Abnormal angiopoietins 1&2, angiopoietin receptor Tie-2 and vascular endothelial growth factor levels in hypertension: relationship to target organ damage [a sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)]. J Intern Med 2005;258:336-43.

[24] Felmeden DC, Spencer CG, Belgore FM, et al. Endothelial damage and angiogenesis in hypertensive patients: relationship to cardiovascular risk factors and risk factor management. Am J Hypertens 2003;16:11-20.

[25] Lim HS, Blann AD, Chong AY, et al. Plasma vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in diabetes: implications for cardiovascular risk and effects of multifactorial intervention. Diabetes Care 2004;27:2918-24.

[26] Chong AY, Caine GJ, Freestone B, et al. Plasma angiopoietin-1, angiopoietin-2, and angiopoietin receptor tie-2 levels in congestive heart failure. J Am Coll Cardiol 2004;43:423-8.

[27] Kiefer FN, Neysari S, Humar R, et al. Hypertension and angiogenesis. Curr Pharm Des 2003;9:1733-44.

[28] Reaven GM. A syndrome of resistance to insulin stimulated uptake (Syndrome X). Definitions and implications. Cardiovasc Risk Factors 1993;3:2-11.

[29] Haffner SM, Stern MP, Hazuda HP, et al. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA 1990;263:2893-8.