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Association of high sensitivity C-Reactive Protein [hsCRP] and Tumour Necrosis Factor-α [TNF-α] with carotid Intimal Medial Thickness in subjects with different grades of glucose intolerance—The Chennai Urban Rural Epidemiology Study (CURES-31)

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Abstract

Objective: To assess the association of high sensitivity C-Reactive Protein [hsCRP] and Tumour Necrosis Factor-α [TNF-α] with IMT in Asian Indians with different grades of glucose intolerance.

Design and methods: Subjects with normal glucose tolerance [NGT]($n=150$), impaired glucose tolerance [IGT] ($n=150$) and type 2 diabetes (DM) ($n=150$) were recruited from the Chennai Urban Rural Epidemiology Study [CURES], in south India. hsCRP was estimated by nephelometry and TNF-α by enzyme linked immunosorbent assay. Carotid IMT was assessed by high resolution B-mode ultrasonography.

Results: hsCRP and TNF-α levels were higher in those with DM [$p<0.001$] and IGT [$p<0.001$] compared to NGT. In linear regression analysis, both hsCRP [$p=0.003$] and TNF-α [$p=0.001$] showed an association with IMT among NGT subjects even after adjusting for age and gender. Among IGT subjects, TNF-α was associated with IMT [$p<0.001$], while no association was observed either with hsCRP or TNF-α in diabetic subjects. In NGT subjects, mean IMT was highest in those with high values [III tertile] of both TNF-α and hsCRP [0.83±0.1 mm; $p<0.001$] followed by those with high TNF-α+low hsCRP [0.74±0.09 mm; $p<0.001$], high hsCRP low TNF-α [0.67±0.09 mm; $p<0.001$], and lowest in those with both low TNF-α and hsCRP [I tertile] [0.63±0.05 mm.

Conclusion: We conclude that in Asian Indians 1. Levels of hsCRP and TNF-α increase with increasing severity of glucose intolerance 2. Both hsCRP and TNF-α are associated with IMT in NGT subjects while TNF-α alone is associated with IMT in IGT subjects 3. hsCRP and TNF-α have a cumulative effect on mean IMT values in NGT subjects.

Keywords: hsCRP; TNF-α; Intimal medial thickness; Glucose intolerance; Diabetes; Asian Indians; South Asians

Introduction

Earlier epidemiological studies have documented that diabetes increases the risk for CAD by 2–4 times [1]. Asian Indians are known to have very high rates of diabetes [2,3] and premature coronary artery disease [4,5]. We have reported that not only is the prevalence of coronary artery disease [CAD] high among subjects with impaired glucose tolerance [IGT] [6], but also, cardiovascular risk factors begin to operate in the IGT phase itself [3].

Insulin resistance [IR], is considered to be one of the important links between glucose intolerance and CAD [7]. Since insulin resistance represents a chronic inflammatory state, recent research studies have focused on the association of inflammatory markers like high sensitivity C-Reactive Protein [hsCRP] and Tumour Necrosis Factor-α [TNF-α] with CAD [8,9]. hsCRP plays a direct role in the formation and rupture of atherosclerotic plaques [10]. Accordingly, elevated levels of hsCRP have been documented in subjects with a history of myocardial infarction [11], stable angina pectoris [12] and acute

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coronary ischemia [13]. hsCRP has also been shown to be strongly associated with various components of metabolic syndrome [MS], a cluster of metabolic abnormalities that contribute to CAD [8,14]. In an earlier study, we have shown that hsCRP is associated with diabetes and CAD [15]. Another inflammatory marker that has gained importance is TNF-α, which plays a key role in mediating insulin resistance by inhibiting insulin receptor activity [16]. Studies in the west have also demonstrated a relation between TNF-α and CAD [17].

However, very few studies have looked at the association of hsCRP and TNF-α with carotid intimal medial thickness [IMT], a preclinical atherosclerotic marker. Earlier studies have demonstrated an independent relationship between hsCRP [18] and TNF-α with IMT [19] in subjects with diabetes but have not looked at the cumulative effect of both on IMT.

As IMT increases with increasing severity of glucose intolerance [20], we thought it is worthwhile to assess the association of IMT with inflammatory markers. Asian Indians have increased prevalence of CAD without excess of conventional risk factors such as smoking, obesity, hypertension, and high cholesterol levels [4–6,21] but with increased levels of newer risk factors like inflammatory [22] and platelet activation markers [23]. In this paper we explore the relation of hsCRP and TNF-α with carotid IMT in Asian Indian subjects with different grades of glucose intolerance.

Research design and methods

The study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiological 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 [24,25]. 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 website 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, California, USA) in all subjects. Subjects were classified as 'known diabetic subjects' if they stated that they had diabetes and were on the treatment.

In Phase 2 of CURES, all the known diabetic subjects (n=1529) were invited to the centre for detailed studies on vascular complications, 1382 responded [response rate 90.3%]. From the rest of the study subjects, 10% of newly diagnosed diabetic subjects [n=320, response rate 98.8%], 15% of subjects with impaired fasting glucose [n=866, response rate 99.1%], and 10% of subjects with normal fasting glucose [n=1494, response rate 97.0%] were recruited. Those who were confirmed by OGTT to have 2 h plasma glucose value ≥11.1 mmol/L [200 mg/dL] based on WHO consulting group criteria [26] were labeled as 'newly detected diabetic subjects'; those with 2 h post glucose value ≥7.8 mmol/L [140 mg/dL] and <11.1 mmol/L [200 mg/dL] [27] as impaired glucose tolerance [IGT] and those with 2 h post glucose value <7.8 mmol/L [140 mg/dL] as normal glucose tolerance [NGT].

Group 1 comprised of 150 healthy normals. Group 2: 150 subjects with impaired glucose tolerance and Group 3: 150 type 2 diabetic subjects. The inclusion criteria were: non-smokers, normal resting 12 lead ECG, absence of angina, myocardial infarction or history of any known vascular, infectious or inflammatory diseases and not on statins or aspirin. Institutional ethical committee approval was obtained for the study and informed consent was obtained from all study subjects.

Measurement of intimal-media thickness

The method used for measurement of carotid IMT at our centre has been described in earlier publications [28,29]. The intima plus medial thickness of the right common carotid artery was determined using a high resolution B-mode ultrasonography system (Logic 400 GE, Milwaukee, U.S.A.) having an electrical linear transducer midfrequency of 7.5 MHz. The axial resolution of the system was 0.3 mm. The images were recorded, as well as photographed. The scanning was done for an average of 20 min.

IMT was measured as the distance from the leading edge of the first echogenic line to the second echogenic line during the diastolic phase of cardiac cycle. Six well-defined arterial wall segments were measured in right carotid system [30]. The reproducibility of the IMT measurement was examined by conducting another scan one week later on 20 subjects by the same sonographer. The mean difference in IMT between the first and second measurements was 0.02 mm, the standard deviation, 0.06 mm and the mean difference ranged between −0.09 mm to +0.09 mm.

Anthropometric measurements

Anthropometric measurements including weight, height and waist measurements were obtained using standardized techniques as detailed elsewhere [24]. The body mass index (BMI) was calculated using the formula, weight (kg)/height (m^2). Blood pressure was recorded in the sitting position in the right arm to the nearest 2 mm Hg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 min apart and the mean of the two was taken as the blood pressure.

Biochemical parameters

Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method) serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method) and HDL cholesterol (direct method–polyethylene glycol–pretreated enzymes) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Glycated haemoglobin (HbA1C) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, Calif., USA).

Quality control checks were done on a daily basis for routine parameters. Our laboratory also participates in the Bio-Rad EQAS programme, the CV for the study parameters [plasma