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Cellular growth factors in relation to mortality from cardiovascular disease in middle-aged Japanese: The JACC study

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ABSTRACT

Objective: Limited evidence has been available on the relationships of cellular growth factors with cardiovascular disease in population-based samples.
Methods: We conducted a nested case–control study under a large prospective cohort study (JACC study) where a total of 39,242 subjects aged 40–79 years provided serum sample. We measured cellular growth factors [insulin-like growth factors I, II and binding protein-3 (IGF-I, IGF-II and IGFBP-3) and transforming growth factor (TGF-β1)] among cases and controls, matched for sex, age, area of residence and year of serum storage.
Results and conclusions: During the follow-up for 9 years, there were 233 deaths from total stroke (49 subarachnoid hemorrhages, 55 intraparenchymal hemorrhages, 71 ischemic strokes), and 97 deaths from coronary heart disease. The multivariable odds ratio (95%CI) of intraparenchymal hemorrhage associated with a 1-SD increment of IGF-I (men:4 8 ng/ml, women: 61 ng/ml) was 0.31 (0.14–0.71). That of ischemic stroke associated with a 1-SD increment of TGF-β1 (men: 8.0 ng/ml, women: 10.9 ng/ml) was 0.58 (0.34–0.98). Serum IGF-II and IGFBP-3 were not associated with mortality from any outcomes. In conclusion, IGF-I was inversely associated with mortality from intraparenchymal hemorrhage while TGF-β1 was so with ischemic stroke, suggesting potential roles of cellular proliferation in the development or prognosis of stroke.

1. Introduction

Cellular proliferation is one of the key components in the large- and small-vessel pathogenesis of atherosclerosis [1] and arteriosclerosis [2] in the development of coronary heart disease (CHD) and stroke. Insulin-like growth factors I and II (IGF-I and -II) are single-chain polypeptides of 70 and 67 amino acids, respectively, that accelerate the proliferation and migration of vascular smooth muscle cells (VSMCs) [3,4]. Further, IGF-I prevents apoptosis of VSMCs, relaxes arteries, and enhances contractility of myocardial cells [3,4].

IGF-binding protein-3 (IGFBP-3) regulates free concentrations of IGF-I and -II (active forms) in the circulatory system to modulate the interaction of IGFs with their receptors on target cells [5]. Transforming growth factor β1 (TGF-β1), another growth factor peptide, may have conflicting effects and accelerate or inhibit the migration and proliferation of endothelial cells and VSMCs, and the overall net effect may be the inhibition of atherosclerosis [6]. However, evidence of the contribution of these growth factors to cardiovascular disease (CVD) is limited.

According to previous cross-sectional studies, higher IGF-I levels are associated with the presence and severity of carotid atherosclerosis [7,8] while lower IGF levels were found among patients with CHD [9,10]. However, several [11–15], but not all [16], prospective studies reported that lower IGF-I levels were associated with increased risk of CHD and stroke. Further, lower IGF-I levels were associated with poor survival among stroke patients [13].

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Lower IGFBP-3 levels were associated with the presence and severity of coronary atherosclerosis [17], and increased risks of CHD [15] and ischemic stroke [14]. Another study showed the opposite finding: lower IGFBP-3 levels were associated with reduced risk of CHD [11]. Lower TGF-β1 levels were associated with risk of cardiovascular deaths among hemodialysis patients [18], and with risk of mortality from non-CVD among patients with CHD [19].

We therefore investigated the associations between these markers and mortality from total stroke, its subtypes, and CHD among middle-aged Japanese people by a nested case–control study under a nation-wide prospective study.

2. Material and methods

2.1. Survey population

We conducted a nested case–control study as part of the JACC study sponsored by the Ministry of Education and Science, which was first conducted in 1988–1990 and involved 110,792 individuals (46,465 men and 64,327 women, age 40–79 years) living in 45 communities throughout Japan who completed self-administered questionnaires about their lifestyles and medical histories of previous CVD and cancer [20]. Informed consent was obtained from the participating individuals when they completed the questionnaire. In several communities, informed consent was obtained at the community level after the study purpose, methods, and data confidentiality had been explained to community leaders and mayors.

A total of 39,242 subjects (35.4% of respondents) provided blood samples after giving individual informed consent. We examined the differences of major cardiovascular risk factors between the subjects with sera and those without sera (Supplemental table). No material differences, although statistically significant due to the large sample size were found between the subjects with sera and those without sera for major cardiovascular risk characteristics except for sex. Men provided the sera more often than women.

After 557 men and 916 women with a history of CVD or cancer at baseline had been excluded, a total of 37,769 subjects (13,282 men and 24,487 women) were enrolled in the study, which was approved by the Ethics Committees of the University of Tsukuba, and Osaka University.

2.2. Mortality surveillance

The participants were followed up to determine mortality of CVD by the end of 1999. For mortality surveillance in individual communities, investigators conducted systematic reviews of death certificates, all of which were forwarded to the public health center in the area of residency. Mortality data were centralized at the Ministry of Health and Welfare, and the underlying cause of death was coded according to the *International Classification of Diseases (ICD)*, 9th revision, for deaths from 1988 to 1994, and the 10th revision for deaths from 1995 to 1999 as used for the National Vital Statistics. Registration of death is required by the Family Registration Law of Japan and is believed to be adhered to throughout Japan. All deaths occurring in the cohort were thus ascertained by death certificates from a public health center, except for subjects who died after moving away from their original community and were treated as censored cases.

The ICD 10th revisions were used to determine cause-specific mortality of CVD as follows: total stroke (ICD 10th revision, codes I60–I69); CHD (I20–I25) and total CVD (I00–I99). Further subgroupings of total strokes were: intraparenchymal hemorrhage (I61), subarachnoid hemorrhage (I60), and ischemic stroke (I63). For each case, one control subject was selected randomly from among participants without mortality from stroke or CHD and matched for sex, age (±5years), community, and year of blood-drawing.

2.3. Determination of biochemical variables

Sera were prepared from blood samples as soon as possible after blood collection at laboratories in or near the surveyed municipalities. The serum from each participant was divided between 3 and 5 tubes (300 μl per tube), and stored at −80 °C until analysis in a single laboratory (SRL, Inc., Hachioji, Japan) [21]. None of the samples had been defrosted. Serum concentrations of IGF-I, IGF-II, and IGFBP-3 were measured by immuno-radiometric assay, using commercially available kits (Daiichi Radioisotope Laboratory, Tokyo). TGFB-1 was measured by sandwich enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN).

All assays were performed and interpreted by individuals who were blinded to the case/control status of samples. The intra-assay precision obtained using different reference sera for each determination method was 2.2–3.5%, 2.7–4.5%, 3.2–4.2%, and 2.7–6.8% of the coefficients variation values for IGF-I, IGF-II, IGFBP-3, and TGF-β1, respectively. The inter-assay coefficient of variance was 1.1–4.2%, 4.2–5.5%, 5.3–8.8% and 4.2–6.2%, respectively.

Serum total cholesterol was measured using the enzymatic method with an automatic analyzer (Hitachi 7600-210; Hitachi Medical Corp., Tokyo, Japan) at Kotobiken Medical Laboratories, Inc. The standardization of lipid measurement was performed with the aid of the Osaka Medical Center for Health Science and Promotion, an international member of the US National Cholesterol Reference Method Laboratory Network (CRMLN) [22].

2.4. Statistical analysis

The Student's *t*-test was used to compare the mean values of baseline cardiovascular risk factors and selected biomarkers for mortality cases and control subjects. The χ^2 test was used to compare percentages of cases and control subjects. The odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for mortality from total stroke, stroke subtypes and CHD according to the sex-specific quartiles and 1-SD increment of serum biomarkers by means of conditional logistic regression models. Cut-off points of the quartile analyses were determined on the distribution of control subjects. Linear regression was employed to test for linear trends across the biomarker categories by using a median variable of biomarker for each biomarker category. Covariates for the adjustment included serum total cholesterol levels (mmol/L: continuous), cigarette smoking status (non and current), current drinking status (non and current), and self-reported histories of physician's diagnosis of hypertension and diabetes mellitus (yes vs. no) as well as matching for sex, age, area of residence and year of serum storage.

All *p*-values for statistical tests were two-tailed and values <0.05 were considered to be statistically significant. The SAS statistical package version 9.1 (Statistical Analysis System Inc., Cary, NC) was used for analyses.

3. Results

During the median nine-year follow-up, we identified 2967 deaths from all causes and 808 deaths from total CVD which included 233 deaths from total stroke (121 males and 112 females), comprising 49 (18 and 31) subarachnoid hemorrhages, 55 (28 and 27) intraparenchymal hemorrhages, 71 (44 and 27) ischemic strokes, and 57 (30 and 27) other strokes, and 97 (53 and 45) CHDs.