YKL-40 Level and Hypertension Incidence: A Population-Based Nested Case-Control Study in China

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Background—Human cartilage glycoprotein-39 (YKL-40) has been suggested to be a new marker of inflammation, atherosclerosis, and endothelial dysfunction. However, whether a higher level of YKL-40 is an independent risk factor for hypertension incidence is still unknown.

Methods and Results—In a nested case-control study within a prospective cohort of 12 423 initially healthy Chinese adults, we measured baseline plasma concentrations of YKL-40 among 700 new-onset hypertension cases and 700 age- and sex-matched controls. Multiple conditional logistic regression analyses were used to calculate the odds ratios (95% CIs) of hypertension associated with higher levels of YKL-40 both in the total population and in the age- (>55 and ≤55 years) and sex-matched subgroups. Among the total population, YKL-40 levels were not associated with hypertension risk. In the subgroup older than 55 years, odds ratios (95% CIs) of hypertension for those in the two higher tertiles of YKL-40 were 1.23 (0.77–1.97) and 1.59 (0.99–2.55) (*P* for linear trend=0.05). In the male subgroup, odds ratios (95% CIs) of hypertension for those in the two higher tertiles of YKL-40 were 1.55 (0.88–2.72) and 2.09 (1.14–3.82) (*P* for linear trend=0.02). An interaction effect was observed between YKL-40 and sex (*P* for interaction <0.01) but not between YKL-40 and age (*P* for interaction=0.21). High YKL-40 level significantly increased hypertension risk in men but decreased hypertension risk with a trend although not significant in women.

Conclusions—This study suggests that YKL-40 is associated with hypertension incidence only among men. The study findings need to be further verified by prospective cohort studies or clinical trials. (***J Am Heart Assoc***. 2016;5:e004534 doi: 10.1161/JAHA.116.004534)

Key Words: high blood pressure • hypertension • nested case-control study • YKL-40

Cardiovascular disease (CVD) is still the leading cause of mortality worldwide. To date, hypertension is regarded as one of the most important modifiable risk factors of CVD. It has been reported that 54% of stroke cases and 47% of ischemic heart disease cases can be attributed to high blood pressure (BP).[1,2] The development of hypertension is a complex process involving many factors.[3] Although the corresponding interventions have improved hypertension control rates,[4] hypertension remains an important public health challenge. Unknown risk factors for hypertension need to be studied to improve hypertension prevention and control.

YKL-40, also known as human cartilage glycoprotein-39 and chitinase-like protein 1, is a 40 kDa heparin- and chitin-binding glycoprotein and a member of the mammalian chitinase-like proteins.[5,6] It is secreted from macrophages, neutrophils, and vascular smooth muscle cells.[7] At present, YKL-40 has been proposed to be a new marker of inflammation, atherosclerosis, and endothelial dysfunction.[8,9] In addition, YKL-40 was found to increase in individuals with hypertension, especially in those with nondipper hypertension.[10,11] However, these investigations were cross-sectional studies without a clear temporal relationship between YKL-40 and hypertension incidence. Therefore, whether YKL-40 can be considered an independent risk factor for hypertension incidence needs to be elucidated. To address this issue, we conducted a nested case-control study in which we measured plasma levels of YKL-40 at baseline in a prospective cohort of initially healthy Chinese adults who were followed up for hypertension incidence.

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Methods

Study Populations

The present investigation was performed as a nested case-control study in 20 343 individuals participating in a population-based investigation of risk factors for CVDs initiated in Changshu, Jiangsu province of China, from 2007 through 2008. These individuals lived in rural communities of south China where the economy situation is relatively developed. They mainly engaged in the agricultural and partly in the handicraft industry. The mean age of participants was 50±14.1 years and 58.1% were women. Data on demographic information, lifestyle risk factors, and personal medical history were collected with standard questionnaires by trained staff. Cigarette smoking was defined as ever having smoked at least 100 cigarettes. Alcohol consumption was defined as consuming any type of alcohol beverage at least 12 times during the past 1 year. Body weight and height were measured using a regularly calibrated stadiometer and balance-beam scale with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the level of 1 cm above the umbilicus.

Participants were advised to avoid alcohol, cigarettes, coffee/tea, and exercise for at least 30 minutes before their BP measurements. Three BP measurements were measured using an electronic BP monitor (Omron HEM-770A, OMRON Healthcare Inc, Dalian, China) with a 30-second interval. BP was measured with the individual in a sitting position after 5 minutes of rest. The mean of the 3 BP measurements was used in the analysis. Hypertension was defined as mean systolic BP ≥140 mm Hg and/or mean diastolic BP ≥90 mm Hg or current use of antihypertensive medications. Individuals were excluded from the current analysis for the following reasons: baseline hypertension or prevalence or history of coronary heart disease, stroke, chronic kidney diseases, tumors, chronic obstructive pulmonary diseases, or peripheral artery diseases. Finally, a total of 12 423 individuals were free of hypertension, CVD, or any other severe disease at baseline.

This study was approved by the institutional ethics review board and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent.

Follow-Up and Outcome Definition

All participants were followed up from April to October 2013 with a face-to-face interview. Data on the personal history of disease and medication during follow-up were obtained using a standard questionnaire written in Chinese and administered by trained staff. Participants who reported an incidence of coronary heart diseases, stroke, chronic kidney diseases, tumors, chronic obstructive pulmonary diseases, or peripheral artery diseases during the follow-up period were asked to provide hospital records, including outpatient, admission records, or discharge summary. Experienced physicians carefully reviewed the records to confirm the above diseases and excluded these patients from the study. Death data were confirmed after obtaining death certificates from the local citizen registry or the attended hospital. The method and equipment for 3 BP measurements were consistent with those at baseline. New cases of hypertension were identified in the following ways: (1) self-reported use of antihypertensive medications in the previous 2 weeks and (2) systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg during the visit. Among 12 423 healthy individuals at baseline, 2344 were lost to follow-up. Finally, a total of 1774 new cases of hypertension were ascertained from baseline to the follow-up end point.

Selection of Cases and Controls

For the present study, we selected new-onset hypertension patients as cases. As controls, we selected normotensive individuals during the follow-up throughout the same time interval as the selected cases. Among 1774 patients with new-onset hypertension, we selected 700 as cases by means of stratified random sampling method according to their resident areas. Controls were matched to the cases 1:1 based on age (birth at the same year) and sex. If more than one control was available, we would select the person living close to the case as the control. The 700 pairs of cases and controls with complete information and blood samples were finally included in this nested case-control study.

Measurements

Blood samples were obtained in the morning by venipuncture after a requested overnight fasting period (at least 8 hours) and sampled in EDTA tubes and immediately spun at 3000 rpm for 15 minutes at study entry. Plasma samples were stored at −80°C and measurements were performed by laboratory technicians who were blinded to the characteristics of the study patients. YKL-40 was reported to be stable in blood samples frozen at −80°C after several cycles of freezing and thawing.[12] Plasma YKL-40 tests were performed using commercial enzyme-linked immunosorbent assay kits (catalog number DC3L10; R&D Systems, Inc, Minneapolis, MN) according to the manufacturer's instructions. A standard curve was constructed from which the YKL-40 concentrations of unknown samples were determined. Intra- and inter-assay coefficients of variation were less than 5% and 7%, respectively.

Statistical Analysis

At baseline, continuous variables were presented as means±SDs and compared by paired-samples *t* test. Categorical variables were presented as number of patients (percentages) and compared by unadjusted conditional logistic regression analysis. Because of the skewed distribution, the concentration of YKL-40 was presented as median with a range between 25th and 75th percentiles. Differences of YKL-40 levels between the cases and controls were compared using paired-sample Wilcoxon signed rank test.

The association of natural log-transformed YKL-40 with hypertension was assessed using multiple conditional logistic regression analysis adjusting for BMI, smoking, drinking, glucose-lowering therapy, lipid-lowering therapy, baseline BP, fasting glucose, and lipid levels. In addition, the study population was divided into tertiles on the basis of the distribution of control values. Odds ratios (ORs) and 95% CIs were calculated for the upper two categories of YKL-40 level with the lowest one as a reference. A test for linear trend was performed by entering categorical variables as continuous parameters in the model. We also set a multiplicative interaction term of YKL-40 and age (>55 or ≤55 years) or sex in the multivariable conditional logistic model to test the interaction effect on hypertension incidence with adjustment for other confounding factors.

We assessed whether the addition of YKL-40 in the multivariable model made better predictions than a model built only based on conventional risk factors using net reclassification improvement (NRI) and integrated discrimination improvement (IDI).[13] NRI represented the incremental ability to accurately reclassify patients with new-onset hypertension into higher risk categories and individuals without hypertension into lower ones after the natural log-transformed YKL-40 level was incorporated into the prediction models. IDI reflected the increase in difference of mean probability to predict hypertension events in cases with that in controls, indicating whether the prediction model with additive natural log-transformed YKL-40 level had a better ability to distinguish cases from controls. Two conditional logistic regression models were applied to estimate the increased discriminative ability based on the YKL-40, and patients were divided into 3 categories for the risk classification (<10%, 10%–30%, and >30%). All statistical tests were 2-tailed and were significant if the *P* value was less than 0.05. Statistical analysis was carried out using SAS software (version 9.3; SAS Institute, Cary, NC).

Results

In Table 1, case and control patients were matched for age (52.6 years on average) and sex (33.3% male). As expected,

Table 1. Baseline Characteristics According to 700 Hypertension Patients and 700 Age- and Sex-Matched Controls

Variable	Patients (n=700)	Controls (n=700)	*P* Value
Age, y	52.6±10.9	52.6±10.9	Matched
Male, No. (%)	233 (33.3)	233 (33.3)	Matched
BMI, kg/m^2	22.7±3.2	21.6±2.8	<0.01
Systolic blood pressure, mm Hg	123±7.8	116±10.5	<0.01
Diastolic blood pressure, mm Hg	76±6.1	72±7.2	<0.01
Total cholesterol, mmol/L	4.6±1.0	4.5±0.9	0.49
Triglycerides, mmol/L	1.5±1.1	1.4±1.0	0.03
Low-density lipoprotein cholesterol, mmol/L	2.5±0.8	2.5±0.7	0.66
High-density lipoprotein cholesterol, mmol/L	1.3±0.3	1.4±0.3	0.02
Fasting glucose, mmol/L	5.1±1.2	5.0±1.0	0.18
Smoking, No. (%)	171 (24.4)	161 (23.0)	0.29
Drinking, No. (%)	138 (19.7)	99 (14.1)	<0.01
Glucose-lowering therapy, No. (%)	5 (0.71)	3 (0.43)	0.48
Lipid-lowering therapy, No. (%)	1 (0.14)	1 (0.14)	1.00

BMI indicates body mass index.

initially healthy persons who subsequently developed hypertension (cases) were more likely to be drinkers; have higher BMI, triglyceride, and BP levels; and lower HDL cholesterol levels compared with those who remained free of hypertension (controls) at baseline. Among the total population and most subgroups, there is no significant difference of YKL-40 levels between cases and controls. However, hypertension patients had a significantly higher level of YKL-40 (69.1 [30–127.2] ng/mL) than controls (55.4 [22.4–111.1] ng/mL) in the male subgroup (Table 2).

As shown in Table 3, YKL-40 was not associated with hypertension incidence among total patients. In the male subgroup, there was a significant association between per-unit increase of log-transformed YKL-40 and hypertension incidence (OR, 1.24; *P*=0.04). In the multivariate analysis, patients in the third tertile had a 1.89-fold increased risk of hypertension compared with those in the lowest tertile (*P*<0.05). ORs of hypertension positively increased with YKL-40 levels (*P* values for linear trend=0.02). After further

Table 2. Comparison of YKL-40 Levels Between Patients and Controls Among the Total Population and Different Subgroups

	Pairs	Patients	Controls	P Value
Total population	700	62.3 (27.3–127.2)	60.8 (25.1–126.6)	0.45
Age, y				
>55	286	87.7 (37.5–151.4)	72.6 (31.1–140.7)	0.25
≤55	414	50.1 (22.1–109)	50.3 (22.4–115)	0.96
Sex				
Male	233	69.1 (30–127.2)	55.4 (22.4–111.1)	0.01
Female	467	58.8 (25.8–127.1)	64.1 (28.7–137)	0.43

adjusting for baseline BP, men in the third tertile still had a 2.09-fold increased risk of hypertension compared with those in the lowest tertile ($P<0.05$). ORs of hypertension also positively and significantly increased with YKL-40 levels (P for linear trend=0.02). In the subgroup of patients older than 55 years, compared with the patients in the lowest tertile, those in the third tertile had a 1.59 increased risk of hypertension with a significant linear trend. However, after further adjusting for baseline BP, this association was not observed. In addition, an interaction effect was observed between YKL-40 and sex (P for interaction <0.01), not between YKL-40 and age (P for interaction=0.21).

With respect to the ability of the prediction model to distinguish hypertension cases from controls, we found that the inclusion of YKL-40 as a predictor conferred discernible improvement in discriminatory performance on models both in the age older than 55 years and male subgroups (Table 4). Among male patients, with those stratified into hypertension risk categories according to the predicted risk probabilities, we found that the prediction model with YKL-40 had a greater ability not only to reclassify hypertension cases into higher risk categories, but to reclassify controls into lower risk categories, relative to the model without YKL-40 (NRI, 41.2%; 95% CI, 23.47%–58.93%; $P<0.01$). Also, the increased performance was further corroborated by the significant improvement in the capability to accurately identify hypertension events in cases rather than in controls after YKL-40 was incorporated into the prediction model (IDI, 0.73%; 95% CI, 0.06%–1.4%; $P=0.03$). A similar association was also observed among individuals aged older than 55 years.

Discussion

In this study, we found that high levels of YKL-40 at baseline predicted hypertension incidence in men. This predictive property is independent of baseline conventional CVD risk factors and improves discrimination between cases and controls beyond these factors.

Table 3. Multivariable Adjusted Odds Ratios (95% CIs) of Hypertension for Upper Tertiles of Plasma YKL-40 Levels Among the Total Population and Different Subgroups

	Log-YKL-40 (per Unit)	Tertile 1	Tertile 2	Tertile 3	P for Linear Trend
Model 1					
Total population	1.04 (0.94–1.14)	1.00 (Ref)	1.24 (0.94–1.64)	1.08 (0.81–1.44)	0.64
Age, y					
>55	1.17 (0.99–1.38)	1.00 (Ref)	1.32 (0.86–2.01)	1.59 (1.02–2.46)	0.04
≤55	0.99 (0.85–1.14)	1.00 (Ref)	1.29 (0.85–1.96)	0.83 (0.53–1.30)	0.94
Sex					
Male	1.18 (0.98–1.42)	1.00 (Ref)	1.48 (0.89–2.45)	1.89 (1.10–3.25)	0.02
Female	0.98 (0.87–1.11)	1.00 (Ref)	0.94 (0.65–1.36)	0.89 (0.62–1.28)	0.94
Model 2					
Total population	1.05 (0.94–1.17)	1.00 (Ref)	1.23 (0.90–1.67)	1.09 (0.79–1.51)	0.62
Age, y					
>55	1.19 (0.99–1.43)	1.00 (Ref)	1.23 (0.77–1.97)	1.59 (0.99–2.55)	0.05
≤55	0.97 (0.86–1.11)	1.00 (Ref)	1.13 (0.79–1.63)	0.86 (0.58–1.27)	0.94
Sex					
Male	1.24 (1.01–1.52)	1.00 (Ref)	1.55 (0.88–2.72)	2.09 (1.14–3.82)	0.02
Female	0.98 (0.86–1.12)	1.00 (Ref)	0.92 (0.61–1.39)	0.87 (0.59–1.30)	0.94

Model 1: adjusted for body mass index, drinking status, smoking, glucose-lowering therapy, lipid-lowering therapy, blood glucose, total cholesterol, triglycerides, and high-density lipoprotein cholesterol. Model 2: Model 1 plus adjustment for baseline systolic and diastolic blood pressure.

Table 4. Comparison Regarding Discrimination Performance Between Multivariate-Adjusted Models With and Without Taking YKL-40 into Consideration Among the Total Population and Different Subgroups

	NRI, %	IDI, %
Total population		
Conventional factors	1.00 (Ref)	1.00 (Ref)
Conventional factors+log-YKL-40	−4.71 (−1.75 to 8.11)	−0.03 (−0.15 to 0.09)
P value	0.47	0.60
Age >55 y		
Conventional factors	1.00 (Ref)	1.00 (Ref)
Conventional factors+log-YKL-40	21.68 (5.41–37.94)	0.66 (0.13–1.18)
P value	0.01	0.01
Age ≤55 y		
Conventional factors	1.00 (Ref)	1.00 (Ref)
Conventional factors+log-YKL-40	3.86 (−9.72 to 17.45)	0.01 (−0.03 to 0.04)
P value	0.58	0.76
Male		
Conventional factors	1.00 (Ref)	1.00 (Ref)
Conventional factors+log-YKL-40	41.2 (23.47–58.93)	0.73 (0.06–1.4)
P value	<0.01	0.03
Female		
Conventional factors	1.00 (Ref)	1.00 (Ref)
Conventional factors+log-YKL-40	7.28 (−5.54 to 20.1)	0.01 (−0.03 to 0.06)
P value	0.27	0.55

Conventional factors included body mass index, drinking status, smoking, glucose-lowering therapy, lipid-lowering therapy, blood glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and systolic and diastolic blood pressure. IDI indicates integrated discrimination improvement; NRI, net reclassification improvement.

Endothelial dysfunction and inflammation are two key steps in the pathophysiology of hypertension.[14] YKL-40, which is produced by macrophages and neutrophils within inflamed tissues, is associated with atherosclerosis and promotes vascular smooth muscle cells attachment, spreading, and migration.[8] Previous studies have detected an association between YKL-40 level and BP. Ma et al[10] reported that patients with either nonmicroalbuminuric (61.63 ng/mL) or microalbuminuric (98.78 ng/mL) hypertension had a significantly higher level of YKL-40 compared with healthy controls (37.85 ng/mL). Bakirci et al[11] further found that YKL-40 level elevation was more obvious among patients with nondipper hypertension. YKL-40 was a good predictor of nondipper hypertension pattern with an area of 0.774 under the receiver operating characteristic curve. However, it is difficult to determine whether the elevation of YKL-40 preceded or was a result of BP rising because of the nonprospective study design used in these studies. In addition, the sample sizes of the above two studies were less than 200 and the statistical power was relatively low. Whether the detected association between YKL-40 and BP could be applied to the total population or certain prespecified

subgroup remains to be further studied. In contrast, our present study is a large sample nested case-control study with high statistical power. The negative association between YKL-40 and hypertension incidence among the total population and the positive association among male patients seems more plausible.

In this study, although there was no difference in YKL-40 levels between the men and the women, the effect of YKL-40 on hypertension development progress is indeed different. As a novel marker of endothelial dysfunction, we speculated that its function was tightly associated with endothelial function of the individuals themselves. Flow-mediated dilation (FMD) is often used as a noninvasive assessment of endothelial function.[15] Skaug et al[16] evaluated the endothelial function by FMD in 4739 healthy adults. Among them, men had a significantly lower FMD than women (4.3±3.9% versus 5.3±4.5%, P<0.01). Lower FMD values in men probably indicate poor endothelial function and substantial prevalence of subclinical atherosclerosis. The sex differences in risk for atherosclerotic events have previously been demonstrated by other studies.[17–19] It has been suggested that sex hormones conserve FMD in women and estrogen played a protective

role in endothelial function.[20,21] In contrast, cigarette smoking aggravates inflammation and endothelial dysfunction in men.[22,23] The significant interaction between YKL-40 and sex in our study further suggests that the more undesirable endothelial dysfunction in men promoted the YKL-40 effect on hypertension incidence.

Study Strengths and Limitations

This study has several strengths that deserve mention. Our study used a prospective nested case-control design in which plasma samples for all study participants were obtained at study entry, a long time interval before the occurrence of the hypertension. Such a study design considerably reduces potential biases inherent in cross-sectional or retrospective studies in which plasma sampling is performed after rather than before the onset of disease. In addition, by matching case and control participants by age and sex and adjusting for additional factors such as blood glucose and lipid levels in multivariable analysis, the present study had a high degree of comparability between cases and controls and controlled for some important confounding factors in the analysis. However, there are also some limitations that should be mentioned. First, the nested case-control design of the study may overestimate the true predictive capacity of the models because of the relative high event rate in case-control studies.[24] Second, all participants were followed up only one time for new-onset hypertension. Therefore, it is difficult for us to estimate the accurate time of onset of new hypertension cases. Furthermore, baseline YKL-40 levels in our study were determined with a single measurement so that the exposure misclassification bias is inevitable but probably low, because a previous study has proved plasma YKL-40 stability over time.[12] Finally, it is unavoidable that there is selection bias because of the nested case-control design. Because of the number of subgroup analyses performed, the statistical significance may have occurred by chance alone.

Conclusions

We found that high levels of baseline YKL-40 predicted hypertension incidence among healthy men. Controlling YKL-40 at a relative low level may be beneficial for hypertension prevention in the male population. The results need to be further verified by prospective cohort studies or clinical trials.

Sources of Funding

Disclosures

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