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Multiple Risk Factor Clustering and Risk of Hypertension in the Mongolian Ethnic Population of China[1]

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Objective To evaluate whether the clustering of risk factors, both environmental and genetic, increases the risk of essential hypertension (EH) and the accumulation of risk factors influences the blood pressure level in normotensives. **Methods** On the basis of a prevalence survey, 501 subjects of Mongolian ethnicity (243 hypertensives and 258 normotensives) who were not related to each other were selected to conduct a case-control study. All subjects were interviewed with questionnaires and their blood specimens were collected. Renin gene insertion/deletion (I/D) polymorphism, a new genetic marker, was genotyped with PCR and polyacrylamide gel electrophoresis. **Results** Overweight, alcohol consumption, and renin gene I/D polymorphism were significant risk factors of EH ($P<0.05$). The odds ratios (*OR*) for the number of risk factors were 2.39 (95%*CI*: 0.98-6.74) for one risk factor, 5.03 (95%*CI*: 2.06-14.18) for two, and 6.09 (95%*CI*: 1.85-22.38) for three respectively after adjusting for age and sex. In normotensives, age- and sex-adjusted mean blood pressures increased with more accumulation of risk factors. However, there were no significant differences among the different blood pressure levels according to the number of risk factors ($P>0.05$). **Conclusion** Overweight, alcohol consumption, and renin gene I/D polymorphism are risk factors of EH in the Mongolian ethnic population of China. The accumulation of the risk factors causes a sharp increase of the risk of EH.

Key words: Essential hypertension; Multiple risk factors; Overweight; Alcohol consumption; Renin gene polymorphism

INTRODUCTION

Essential hypertension (EH) is an important risk factor for cardiovascular diseases. At present, EH is thought to be a polygenic and multifactorial disease, resulting from the interaction between genetic and environmental factors. Epidemiological studies have shown that some environmental factors play roles in the development of EH, such as excessive salt intake, alcohol consumption and overweight. On the other hand, the role of genetic factors have been indicated through the studies of familial aggregation, rates concordance among monozygotic and dizygotic twins and animal models of hypertension[1-5]. The information regarding the relationship between the number of risk factors and hypertension is very limited. The present study is therefore designed to evaluate the link between the number of risk factors and the risk for hypertension and between the accumulation of risk factors and blood pressure levels in normotensives.

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MATERIALS AND METHODS

Subjects

On the basis of a prevalence survey that was performed at the pastoral area of Tongliao, Inner Mongolian Autonomous Region in 2002, 501 subjects (243 hypertensives and 258 normotensives) who were not related to each other were selected to conduct a case-control study. The subjects were all Mongolians who had lived in this area for at least three generations and were 30 years of age or older. All subjects were interviewed with a questionnaire including socio-demographic data, disease history, smoking habits, alcohol consumption, family history of hypertension, anthropometry, and had their blood pressures measured. Informed consent was obtained from each subject.

Using a standard mercury sphygmomanometer, blood pressure (BP) was measured on the right arm of the subjects in a sitting position and resting for at least 10 minutes. The mean values of three measurements were used as the values of BP for the subjects. The criteria for hypertension were defined as systolic blood pressure (SBP) higher than or equal to 140 mm Hg, and/or diastolic blood pressure (DBP) higher than or equal to 90 mm Hg. None of the subjects received any antihypertensive treatment. The subjects with diabetes or evidence of secondary cause of hypertension were excluded. Height and weight was measured to calculate body mass index (BMI) as (weight in kg)/(height in m)^2. Overweight was defined as BMI$\geqslant$24 kg/m^2. Their history of alcohol consumption and tobacco use was recorded. A cigarette smoker was defined as smoking at least one stick per day for no less than one year. An alcohol consumer was defined as drinking at least 50 g of alcohol per day for no less than one year. Venous blood was sampled for the measurement of biochemical indicators and DNA extraction.

DNA Analysis

DNA was extracted from peripheral blood leukocytes according to usual methods[6]. A new genetic marker of human renin gene, an insertion/deletion (I/D) polymorphism of 6-8 "A" of intron 1, was analyzed by polymerase chain reaction (PCR) and nondenaturing polyacrylamide gel electrophoresis. The oligonucleotide primers were designed. The sequence of the forward primer was 5'ACTGACCCACTGTGCTCT 3', and the sequence of the reverse primer was 5'CCTGTGACACCCTAAGCA 3'. The PCR protocol was as follows: initial denaturation at 94℃ for 3 min, followed by 25 cycles of denaturation at 94℃ for 50 s, annealing at 56℃ for 50 s and extension at 72℃ for 50 s with a final extension at 72℃ for 5 min. PCR products were run in 10% nondenaturing polyacrylamide gel electrophoresis at 250 volts and 20℃ for 5 hours, and stained by $AgNO_3$. Three kinds of genotypes were identified as DD, ID, and II.

Statistical Analysis

All statistical analysis was conducted using SAS version 6.12 for Windows. The data were expressed as $\bar{x} \pm s$ or proportion. Differences in quantitative data between hypertensive and normotensive subjects were examined by student's *t*-test. Differences in categorical data between the hypertensive and the normotensive subjects were examined by χ^2 test. The odds ratio (*OR*) of hypertension was estimated with a logistic regression model. Statistical significance was defined as $P<0.05$.

Variables and Assignment

Age was divided into four groups 30, 40, 50, and 60 years of age with an assignment of 0, 1, 2, and 3. Female and male were assigned as 0, 1. BMI<24 and $\geqslant$24 were assigned as 0, 1. Non-smoking and smoking were assigned as 0, 1. Non-drinking and drinking were assigned as 0, 1. Renin gene I/D polymorphism genotype II and (ID+DD) were assigned as 0, 1.

RESULTS

Characteristics of Hypertensive and Normotensive Subjects in Terms of Their Demographic and Environmental Factors

There were significant differences in age, sex, SBP, DBP, BMI, and alcohol consumption between the hypertensive and normotensive subjects. No significant difference in cigarette smoking was observed between them (Table 1).

Analysis for OR of Hypertension Risk Factors

A multivariate logistic regression model was used, which included age, sex, BMI, cigarette smoking, alcohol consumption, and the renin gene I/D polymorphism. The analysis showed that age, BMI, alcohol consumption, and the renin gene I/D polymorphism were statistically significant variables. The *OR* of renin gene genotype (ID+DD) to renin gene genotype II on hypertension was 1.92 (*OR* 95%*CI* 1.09-3.48) (Table 2).

To estimate the effects of risk factor clustering on hypertension, two sets of logistic regression models were used with the cumulative number of risk factors as the main predictors; an unadjusted model and an adjusted model that included age and sex as covariates. The analysis showed that the *OR* of

TABLE1

Characteristics of Hypertensive and Normotensive Subjects

Variables	EH[a]	NT[b]	*P*
Number	243	258	
Age (year)	53.27±11.28	50.33±9.54	0.0018
Sex (% male)	40.33	29.46	0.011
SBP (mmHg)	159.35±24.20	119.60±10.77	0.0001
DBP (mmHg)	98.29±11.29	78.10±6.23	0.0001
BMI (kg/m^2)	23.20±4.12	22.07±3.16	0.0007
Alcohol Consumption (%)	38.68	27.13	0.006
Cigarette Smoking (%)	49.38	46.51	0.52

Note. [a]EH, essential hypertension; [b]NT, normotensive.

TABLE 2

Multivariate Adjusted *OR* of Hypertension Risk Factors

Risk Factor	β	Std Err	Wald χ^2	*P*	Standardized β	*OR*	95%*CI*
Age	0.2887	0.1055	7.50	0.0062	0.144542	1.34	1.09-1.64
BMI	0.8393	0.2085	16.21	0.0001	0.213341	2.32	1.54-3.50
Alcohol Consumption	0.6011	0.2060	8.51	0.0035	0.155671	1.82	1.22-2.74
Renin I/D	0.6539	0.2949	4.92	0.0266	0.118005	1.92	1.09-3.48

Note. [a]*OR*, odds ratio; [b]*CI,* confidence interval.

hypertension increased with the increasing number of risk factors. The relationship between the number of risk factors and the *OR* of hypertension was not altered after adjusting for age and sex (Table 3).

TABLE 3

OR for Hypertension Based on the Number of the Risk Factors

Number of Risk Factors	*n*	*OR*[a] (95%*CI*[b])	
		Unadjusted Model	Adjusted Model[c]
0	29	1.00	1.00
1	211	2.44 (1.01-6.82)	2.39 (0.98-6.74)
2	237	5.53 (2.30-15.43)	5.03 (2.06-14.18)
3	24	6.39 (1.97-23.18)	6.09 (1.85-22.38)

Note. [a]*OR*, odds ratio; [b]*CI*, confidence interval. [c]Adjusted for age and sex.

Prevalence of Cumulative Number of Risk Factors

The prevalence rates of the cumulative number of risk factors in hypertensive and normotensive subjects were different. Only 2.47% of the hypertensive subjects were free from these three risk factors, compared with 8.91% of the normotensive subjects. The proportion of hypertensive subjects that simultaneously had two or three risk factors was 1.5-fold (57.61% versus 37.60%) or 1.8-fold (6.17% versus 3.49%) greater than that of the normotensive subjects (Fig. 1).

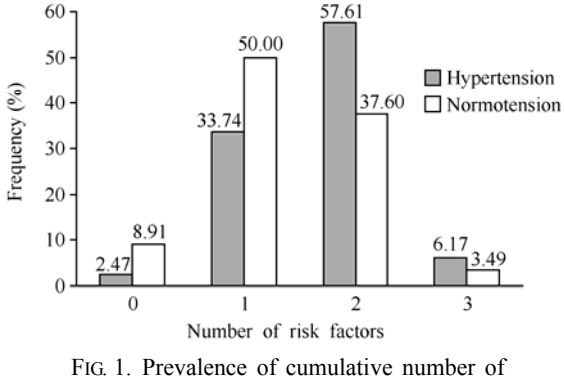

FIG. 1. Prevalence of cumulative number of risk factors in hypertensive and normotensive subjects.

Mean Blood Pressures according to the Number of Risk Factors in Normotensive Subjects

The mean blood pressure increased with the

accumulation of risk factors in normotensive subjects, although both of mean SBP and DBP in those without all the three risk factors were not significantly different from those with one, two or three risk factors. The increasing trend was not altered after adjusting for age and sex. The mean SBP and DBP were the highest with all the three risk factors in the normotensive subjects (Fig. 2).

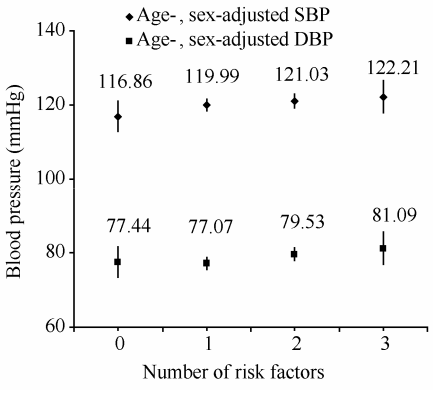

FIG. 2. Age- and sex-adjusted mean blood pressures, according to the number of risk factors in normotensive subjects. Error bars indicate SE.

DISCUSSION

Many studies have proven that some environmental factors play important roles in the development of EH[7-11]. This study shows that overweight and alcohol consumption are risk factors for hypertension. The results presented also support the previous findings that the renin gene I/D polymorphism of intron 1 may be a genetic predisposing factor for EH in the Mongolian ethnic population of China. Overweight, alcohol consumption, and renin gene I/D polymorphism all play independent roles in hypertension. Each factor can increase the risk of EH.

Overweight and obesity are important risk factors for EH and are also independent risk factors for the development of coronary heart disease and strokes. WHO reported that the proportion of hypertension caused by obesity was 30%-65% in western countries. A population-based survey[12] discovered that over 50% of hypertensives were overweight. In the present study on the Chinese Mongolian population, we have also identified that BMI is associated with EH. Overweight can increase the risk of EH.

Many studies have confirmed the relationship between alcohol consumption and EH. Klag[13] reported that the amount of alcohol consumption was positively correlated with blood pressure. Alcohol consumption is also correlated with the prevalence of EH. This suggests that alcohol is an independent risk factor for EH. The studies in Beijing and Guangzhou of China have shown that male drinkers have 40% higher risk of EH than non-drinkers during the 4 year period of time. The present study also shows that alcohol consumption plays a role in the development of EH. Mongolian people traditionally have the habit of drinking large volumes of alcohol which helps to clarify the relationship between alcohol consumption and EH. The analysis shows that the *OR* of drinkers to non-drinkers on EH is 1.82 (*OR* 95%*CI* 1.22-2.74).

The human renin gene is an important candidate in the genetics and molecular epidemiology of EH. (1) Renin is the limiting enzyme in controlling the production of angiotensin II in renin-angiotensin system (RAS). (2) Renin gene has been shown to influence blood pressure and has a close association with the development of EH in some animal models[3,14]. (3) Blockade of the renin-angiotensin system is highly effective in treating most of the hypertensive patients as illustrated by angiotensin I-converting enzyme inhibitors. In the present study, the human renin gene I/D polymorphism of 6-8 "A" of intron 1 was analyzed. Multivariate logistic regression model has shown that the renin gene I/D polymorphism is a statistically significant variable for EH. The renin gene genotype DD or ID can increase genetic susceptibility of EH. The *OR* of the renin gene genotype (DD+ID) to genotype II is 1.92 (*OR* 95%*CI* 1.09-3.48).

In assessing the relative risk of EH through accumulation of three risk factors; overweight, heavy alcohol consumption and the renin gene I/D polymorphism, it is revealed that the accumulation of these factors imposes higher risks than a single risk factor for EH. The *OR* of EH sharply increased when having two or more risk factors. For normotensive subjects, accumulation of risk factors influences blood pressure. The mean blood pressures (SBP, DBP) increase with the accumulation of the risk factors, although no significant difference is observed. This is possibly due to too few normotensive subjects. A study with a larger sample should be carried out in the future. In this study, only three risk factors for EH were analyzed. Accumulation of more risk factors may lead to continuous increase of blood pressure levels in normotensive subjects and their blood pressure levels may reach the threshold of hypertension. The differences in the prevalence of the cumulative number of risk factors between hypertensive and normotensive subjects reveal that hypertensive subjects have been exposed to more risk factors. Tozawas *et al*.[15] have also found that the

clustering of risk factors is significantly associated with EH, and the number of risk factors are positively correlated with the blood pressure levels in non-hypertensive subjects. It is important to recognize and identify multiple risk factors among the normotensive subjects for the prevention of EH.

The relationship between the accumulation of risk factors and EH indicates that the more risk factors, the more risk to EH. Blood pressure increases along with the increasing number of risk factors in normotensive subjects. The accumulation of the risk factors is positively associated with EH. We can conclude that clustering of risk factors plays an important role in the pathophysiology of EH. It constitutes a critical step to prevent EH to recognize and avoid risk factors, especially multiple risk factors.

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