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Racial differences of endothelial function and plasma endothelin 1 level in preclinical Tibetan and Han male population

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Abstract. – **OBJECTIVE: The differences in endothelial function between Tibetan and Han nationality population have not been fully investigated. The aim of this work is to investigate the differences in endothelial function and plasma endothelin 1 (ET-1) concentration between Tibetan and Han male population.**

PATIENTS AND METHODS: Totally 272 Tibetan male subjects aged 42.9 ± 9.4 years were enrolled in this study to stand for Tibetan population. All of them were native residents in Lhasa City. And 580 Qinghai-Tibet Railway constructors with Han nationality aged 41.8 ± 11.1 years were enrolled in this study to stand for Han nationality population. All of them were male subjects and lived in Lhasa City for at least 1 year. All subjects lived in the same high-altitude area (the altitude of Lhasa is 3658 m). Height, weight, waist circumference, hip circumference, systolic blood pressure, diastolic blood pressure were evaluated. Body mass index (BMI) was calculated. Brachial artery flow-mediated dilation (FMD) was measured in the fasting state using high-resolution B-mode ultrasound. Computer-assisted analysis software was used to calculate brachial artery diameters. Venous blood was sampled for the measurement of total cholesterol (CH), triglyceride (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and HbA1c. Plasma ET-1 was quantitated using a commercially available ELISA kits.

RESULTS: Totally 272 Tibetan subjects and 580 Han nationality subjects were enrolled in this study. BMI and waist-hip ratio in Tibetan subjects were much higher than those in Han subjects ($p < 0.01$). LDL cholesterol level and plasma ET-1 concentration in Tibetan subjects were significantly higher than Han subjects ($p < 0.01$). The baseline brachial artery diameter in Tibetan group was much higher than that of Han group ($p < 0.01$). The absolute and percent changes in brachial artery diameter were lower in Tibetan population compared with Han population ($p < 0.01$). The linear regression analysis showed that LDL cholesterol, and plasma ET-1 were correlated with FMD ($p < 0.01$).

CONCLUSIONS: Endothelial function and plasma ET-1 concentration were different between Tibetan and Han male populations.

Key Words:

ET-1, Endothelial function, Preclinical, Tibet, Racial difference.

Introduction

Traditional risk factors for atherosclerotic vascular disease (ASVD) have been assessed intensively to select subjects for appropriate investigation and treatment. But many subjects at high risk of coronary artery disease do not have these traditional risk factors, such as hypertension, dyslipidemia, metabolic disorders[1]. New markers of atherosclerotic risk have been explored. In recent two decades, endothelial function has been suggested as an independent marker of ASVD[2,3]. Evidence from clinical trial suggests that endothelial dysfunction may play an important role in the pathogenesis of cardiovascular disease (CVD) and is associated with an increase in cardiac events[4]. Racial variations have been reported in the endothelial function among African American and Caucasian[5]. Our previous studies revealed endothelial dysfunction in Tibetan young adults[6], even in adolescents[7]. To the best of our knowledge, the difference of endothelial function between Tibetan population (which is a minority in south-west China, about 0.2% of the total Chinese population) and Han population (which is the majority in China, more than 90% of the total Chinese population) is still unknown. The community-

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based prospective study was designed to investigate the difference in endothelial function between Tibetan and Han population.

Patients and Methods

Patients

The Human Ethics Committee of our institute approved the research protocols in accordance with international agreements (Helsinki Declaration[8] of 1975, revised 2008). All participants obtained written informed consent. The participants were recruited through workers who built Qinghai-Tibet Railway in high altitude areas, all of them are young to middle-aged adults between 30 to 50-year-old with male gender. Totally 272 Tibetan male subjects were designed to be representative of the Tibetan population. All of the Tibetan subjects were native Tibetan, who were born in and grown up in Tibet with Tibetan nationality. Another 580 male workers were recruited to be representative of the Han nationality population. All of the Han subjects came from Si-chuan province (neiborhood of Tibet) and had been in Lhasa for more than 1 year. Participants with a history of Raynaud's phenomenon were excluded.

Basic Characters

All participants provided a medical history and underwent a clinical examination at baseline, which mainly included measurement of height, weight, waist and hip circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP). BMI was measured as the ratio between weight and the square of the height. Cigarette smoking: never smoke scored as 0, quitted as 1, always smoking as 2. The "always smokers" were included to analyze smoke rate.

Laboratory Methods

Biochemistry assay. Venous blood was sampled after a 12-hour overnight fasting for the measurement of total cholesterol (CH), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and HbA1c. The laboratory determinations were carried out with Hitachi 7600 automated biochemical analyst (Tokyo, Japan).

Quantitation of plasma ET-1. For plasma ET-1, 10 ml of venous blood were collected into an EDTA tube and centrifuged immediately at 2,500 g for 20 min at 4°C. ET-1 was quantitated using a commercially available ELISA kits (Morinaga, Irvine, CA, USA and R&D System, Minneapolis, MN, USA), as described previously[6]. Standards, reagents, and test samples were prepared and assayed according to the instructions of the manufacturer.

Endothelial Function by Ultrasound Measurements

FMD (Flow-Mediated Dilation)

Measurement of brachial artery flow-mediated vasodilation. Left brachial artery diameter was measured from B-mode ultrasound images at rest and during reactive hyperemia. All participants were examined supine after 20 minutes' rest, and in the fasting state. The room temperature was about 20°C. In the left arm, a sphygmomanometer cuff was positioned 5 cm below the antecubital fossa. A 10-MHz transducer (Vivid 7, GE Corporation, Fairfield, CT, USA) was used to image the left brachial artery. Briefly, a resting scan was performed and arterial flow velocity was measured using a Doppler signal. Increased flow was then induced by inflation of an appropriately sized blood pressure cuff around the forearm to a pressure of 250 mmHg for 4.5 minutes, followed by release. The brachial artery was then imaged during cuff inflation and 2 minutes after cuff release. After release the cuff, reactive hyperaemia occurs, that is, flow in the brachial artery increases to accommodate the dilated resistance vessels in the forearm. A second scan was taken continuously 40 to 180 seconds after cuff deflation. The percentual dilation from baseline in 10 second intervals was assessed. From these data, peak FMD was determined. All the measurements were performed by a single sonographer to ensure the reliability of the data. The sonographer was blinded to the allocation groups. Computer-assisted analysis software was used to calculate brachial artery diameters.

Statistical Analysis

All analyses were carried out with Stata 7.0 software system. Data were expressed as mean±standard deviation. Analysis of variance (ANOVA) and Student's *t*-test was used to evaluate the measurement data. Chi square statistic was used to compare count data. The linear regression analysis was used to evaluate the relationship between risk factors and FMD. Statistical significance was inferred at $p < 0.05$.

Table I. Characteristics of the study population.

	Han nationality	Tibetan nationality	t (χ^2)-value	p-value
Age (years)	41.8 ± 11.1	42.9 ± 9.4	1.4137	0.1578
SBP (mmHg)	132.5 ± 18.1	133.2 ± 14.7	0.5574	0.5774
DBP (mmHg)	79.6 ± 11.2	81.1 ± 9.1	1.9300	0.0539
HbA1c (%)	5.76 ± 0.78	5.82 ± 0.87	1.0082	0.3136
Total cholesterol (mmol/L)	5.37 ± 1.05	5.42 ± 0.97	0.6637	0.5071
HDL cholesterol (mmol/L)	1.21 ± 0.37	1.20 ± 0.33	0.3804	0.7038
LDL cholesterol (mmol/L)	3.07 ± 0.86	3.49 ± 0.91	6.5223	< 0.001
Triglyceride (mmol/L)	1.97 ± 1.44	2.31 ± 1.31	3.4200	0.0007
BMI (kg/m^2)	26.5 ± 3.4	30.1 ± 2.5	15.5951	< 0.001
Waist-hip ratio	0.88 ± 0.05	0.92 ± 0.07	9.5254	< 0.001
ET-1 (pg/ml)	6.57 ± 1.47	8.11 ± 2.15	12.2097	< 0.001
Baseline brachial diameter (mm)	4.03 ± 0.04	4.28 ± 0.06	71.9156	< 0.001
Hypertension, n (%)	103 (17.8)	58 (21.3)	1.5354	0.2150
Diabetes, n (%)	48 (8.3)	28 (10.3)	0.9284	0.3350
Smoke rate, n (%)	492 (84.8)	243 (89.3)	3.1801	0.0750
Smoke	1.78 ± 0.56	1.85 ± 0.46	1.7966	0.0727

Values are mean ± SD, unless otherwise indicated. All subjects are male. Han nationality: 580 subjects, Tibetan nationality: 272 subjects.

Results

As shown in Table I, there were no statistical differences between groups in age, SBP, DBP, CH, HDL-C, HbA1c, and prevalence of hypertension and diabetes ($p > 0.05$). The LDL-C and triglyceride level in Tibetan group were higher than those in Han group ($p < 0.001$). BMI and waist-hip ratio of Tibetan subjects were much higher than those in Han nationality subjects ($p < 0.001$), which indicated that abdominal obesity was prominent in Tibetan subjects. Comparison of plasma ET-1 concentrations showed much higher ET-1 level in Tibetan than Han subjects.

Tibetan population had a higher baseline brachial artery diameter ($p < 0.001$) (Table I). The absolute and percent changes in brachial artery diameter were lower in Tibetan population compared with Han population ($p < 0.001$) (Table II).

The linear regression analysis showed that LDL cholesterol and ET-1 were correlated with FMD ($p < 0.001$), which is an indicator for endothelial function (Table III).

Discussion

A number of studies have suggested that the endothelial dysfunction was an independent predictor of cardiovascular disease[9]. Flow-mediated dilatation (FMD) is the most widely used non-invasive ultrasound method to assess endothelial function, which can provide accurate and reproducible data[10]. The measurement of endothelial function in the conduit arteries of the peripheral circulation by FMD enables study of the key aspects of vascular biology in preclinical subjects in whom invasive testing is not feasible. Over the last 20 years, technical modifications and the development of analysis software have greatly improved image acquisition and reduced method variability. A number of patient related and environmental factors have been shown to influence FMD, including mental stress, food, drugs, and temperature. Recent data, however, from population studies, demonstrate that the contribution of environmental factors to the variability of FMD is relatively small, and these should not be considered limiting factors for FMD assessment[11]. FMD of the brachial artery has been shown to be

Table II. Changes in brachial artery FMD.

	Han nationality	Tibetan nationality	t-value	p-value
Absolute change (mm)	0.141 ± 0.006	0.124 ± 0.005	40.5820	< 0.001
Percent change (%)	3.587 ± 0.152	2.934 ± 0.204	52.1732	< 0.001

Table III. Linear regression analysis for the correlation of FMD and risk factors.

FMD	*t*-value	*p*-value
LDL cholesterol	5.17	< 0.001
ET-1	4.21	< 0.001

Values are mean ± SD, unless otherwise indicated. All subjects are male. Han nationality: 580 subjects, Tibetan nationality: 272 subjects.

the result of endothelium-derived nitric oxide (NO) release and is related to coronary vasoreactivity. FMD has proven to be predictive for the presence of coronary artery disease, for future cardiovascular events, and for postoperative cardiovascular events in high-risk populations[12].

Different clinical centers showed contradictory racial variance in endothelial-dependent forearm vascular resistance or brachial artery dilatation[5,13]. To the best of our knowledge, little has been done to evaluate racial difference of endothelial function between Tibetan (minority in China) and Han (the overwhelming majority in China) population. To exclude the possible interference of some complex factors, only male subjects were recruited to the study. All subjects had similar physical activity and working environment. Also the nature of work of the examined subject were similar. All the Tibetan subjects came from communities in Lhasa city. In order to be consistent with the control group (Han nationality), all the Tibetan subjects aged from 30-50 years. All subjects were screened from railway builders, who are young-middle male persons aged from 30-50 years. That is, the two groups were age and sex matched. All of them lived in the same altitude (3658 m above sea level). The results of this study revealed that Tibetan subjects had significantly reduced brachial artery FMD compared to Han nationality subjects. It is assumed that these differences are the result of genetic factors that differ by race. Tibetan population may be at higher CVD risk. Poor endothelial function may play a role in atherogenesis in Tibetan population.

In this work, although all subjects were age-matched and recruited from the similar working environment with similar physical activity, we found the Tibetan subjects were overweight. The BMI and waist-hip ratio of Tibetan subjects were significantly higher than Han subjects. The most possible reason is the difference of dietary pattern between Tibetan and Han population. Tibet is a high-altitude area in south-west China. The native residents are Tibetan population, which is a minority with their special traditional life habit. The intakes of protein and fat in Tibetan people were significantly higher than people of other areas, mainly due to the consumption of Zanba (which consists of Tibetan naked barley, Tibet salt cream tea, and Tibetan cream). The traditional Tibetan food is rich of protein and fat, but poor of fruit and vegetables[14]. Our previous study revealed the poor health education and compliance of doctor's order[15]. For example, the smoke rate of Tibetan male undergraduate student is 70.4%, most of them believed "smoke is no harm to health", and never think of give-up smoking[16].

A large amount of investigations have shown that total cholesterol and LDL cholesterol are related with impaired endothelial function. The adverse effect of cholesterol on endothelial function was reported in young subjects, even in children. Study found that endothelial impairment correlated with LDL cholesterol level in children with familial hypercholesterolaemia from as early as around 10 years of age[17]. Our report showed LDL cholesterol in Tibetan subjects was significantly higher than that in Han subjects. This finding was consistent with our previous studies among Tibetan population [18]. We suppose that the comparatively higher LDL cholesterol level play a role in endothelial function impairment among Tibetan population. However, it remains unclear whether high LDL cholesterol level is ethnic related or not.

In the present study, we investigated plasma ET-1 concentration in Tibetan and Han male subjects. We found ET-1 levels in Tibetan to be much higher than in Han subjects. Both of Tibetan and Han subjects we studied were living in the same high altitude area and had similar physical activity. To the best of our knowledge, few researches showed the racial differences in plasma ET-1 concentrations between Tibetan and Han populations. Since ET-1 has potent contractile and proliferative properties, it has been suggested to be involved in the pathogenesis of endothelial dysfunction and atherosclerotic diseases[19,20]. ET-1 has been proposed to modulate endothelial function through several pathways. ET-1 exerts its contractile effects via endothelin-A receptors on vascular smooth muscle cells, and it also causes vasodilation via nitric oxide release mediated by endothelin-B receptors on endothelial cells. In addition, ET-1 interacts with nonendothelial pathways, eg, the sympathetic system, renin-angiotensin system, and central nervous system[21]. Evidence has demonstrated that ET-1 is involved in the pathogenesis of endothelial dysfunction and regulation of vascular tonus[22]. In our study, the higher plasma ET-1 concentration in Tibetan subjects than in Han subjects may partly explain the deteriorated endothelial function in Tibetan. Given the fact that ET-1 induces long-lasting vasoconstriction and modulates the sympathetic system–mediated contractility, it is likely that ET-1 contributes to the abnormal vascular reactivity in Tibetan.

Tibet is a developing high-altitude area in south-west China. We concerned about the public health of Tibetan population because of the poor environmental situation including nutrient and health education. We concerned about the situation, that's why we recruited Tibetan subjects into the study and compared with the age and sex-matched Han subjects. Except the traditional risk factors for endothelial dysfunction (such as LDL cholesterol), we found plasma ET-1 concentration in Tibetan population were also significantly higher than the Han controls. It is not clear the high plasma ET-1 concentration is racial related or not. The precise rationale for the deteriorated endothelial function in Tibetan population remained unclear.

The present study revealed the differences in endothelial function between Tibetan and Han populations. But there are several possible limitations to these analyses. First, there could be other CVD risk factors that both affect endothelial function and differ by race that were not accounted for. Second, only young-middle aged male subjects were recruited in this study. The reason why we find such a group of participants is that it is easy to find a large sample in railway builders. The baseline characteristics of the control group (Han nationality) were very similar. All the railway builders are young-middle aged male subjects. Therefore, it is not clear to what extent these findings can be extrapolated to the general public.

In summary, the racial differences in endothelial function remain largely unexplained. Brachial artery FMD was lower in Tibetan male subjects compared to Han nationality subjects. Future questions should address whether endothelial dysfunction exists in general Tibetan population. Can it predicts future cardiac events? Whether Tibetan population can be benefit from interventions (such as statins) designed to improve endothelial health?

Conclusions

Endothelial function and plasma ET-1 concentration were different between Tibetan and Han male populations.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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